ARE CYCLIC AMP EFFECTS RELATED TO REAL PHYSIOLOGICAL PHENOMENA ?

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Received January 25, 1974

SUMMARY - In spite of the fact that cyclic AMP and enzymes involved in its metabolism are absent from Bacillus megaterium *(1), this organism still exhibits catabolite repression which can be largely relieved by cyclic AMP.*

It is currently believed that catabolite repression in bacteria is mediated by cyclic AMP and a protein factor that specifically binds cyclic AMP. Most authors interpret catabolite repression as dependent on a regulation of the intracellular level of cyclic AMP.

It has been recently shown (1) that *Bacillus megaterium* does not contain detectable amounts of cyclic AMP ($<10^{-9}$M). Adenyl cyclase and cyclic AMP phosphodiesterase appear also to be absent in this organism. Therefore it seemed of interest to investigate the effect of cyclic AMP on catabolite repression in *Bacillus megaterium*.

Materials and Methods.

Bacillus megaterium strain MA102 was grown in minimal salt medium as described by Aubert and Millet (2) in the presence of 0.2 % of a carbon source. The bacteria were grown at 30°C. β-galactosidase was induced with 10^{-2}M galactose. After 3-4 generations, cell growth was arrested with 50 µg/ml chloramphenicol and the suspension was sonicated. β-galactosidase was assayed as described previously (3).

Results.

Table I shows the differential rate of β-galactosidase synthesis in glucose, glycerol and galactose as carbon sources and the effect of cyclic

T A B L E I

DIFFERENTIAL RATE OF β-GALACTOSIDASE SYNTHESIS

IN *BACILLUS MEGATERIUM*

Carbon source	Addition	U/mg β-galactosidase	Ratio $\frac{+cAMP}{-cAMP}$
Glucose	-	9.3	
	+cAMP	27	2.9
	+5'AMP	8.8	
Glycerol	-	79	
	+cAMP	280	3.6
	+5'AMP	73	
Galactose	-	210	
	+cAMP	810	3.9
	+5'AMP	220	

TABLE I.

Cultures were grown and induced as described in *Materials and Methods*. Generation time in glucose : 85 min. ; in glycerol : 100 min. ; in galactose : 135 min. Cyclic AMP and 5'AMP were used at a final concentration of 9 mM.

AMP and 5'AMP on the rate of synthesis. It can be seen that β-galactosidase synthesis is about eight times higher in glycerol and about twenty times higher in galactose than it is in glucose. Therefore it can be concluded that enzyme synthesis is under the control of catabolite repression. Cyclic AMP stimulates the rate of enzyme synthesis by a factor of 3 to 4 independently of the nature of the carbon source. The effect seems to be specific for cyclic AMP.

Figure 1 shows the concentration dependence of the cyclic AMP effect.

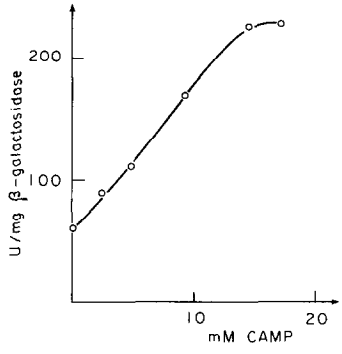

Figure 1.

Differential rate of β-galactosidase synthesis as a function of cyclic AMP concentration. The carbon source is glycerol and the generation time is 80 minutes (independent of the presence or absence of cyclic AMP).

Maximal effect is obtained at about 10 mM cyclic AMP which may appear fairly high although, in fact, it is only about twice the concentration giving maximal effects in *Escherichia coli*. It has to be noted that even at 17 mM cyclic AMP bacterial growth is not affected.

Discussion.

Even though it appears quantitatively quite significant, the effect of cyclic AMP in relieving catabolite repression in *Bacillus megaterium*

cannot be considered "physiological", since this organism appears to lack cyclic AMP as well as adenyl-cyclase. We should therefore conclude that cyclic AMP in fact interferes in some way with the normal control, which must involve mediators other than cyclic AMP.

A possible candidate for a mediator whose effects might be interfered with by cyclic AMP, could be cyclic GMP. The presence of this nucleotide in *Bacillus megaterium* has not been systematically investigated. However the test for cyclic AMP might have also revealed the presence of cyclic GMP, if its level in the cells had been of the order of 10^{-7}M or more.

Whether or not cyclic GMP or some other compound is actually involved in the normal control of catabolite repression in *Bacillus megaterium*, our results illustrate the fact that to observe an effect of cyclic AMP (whether *in vivo* or *in vitro*) upon enzyme synthesis in an organism, cannot be taken as proof of its normal involvement in this type of control. At this point, it seems relevant to mention a number of recent observations which are difficult to reconcile with the view that intracellular levels of cyclic AMP constitute the unique "controller" of catabolite repression, even in those systems where its effects have been most actively investigated. Among these observations, the following appear particularly significant.

1. In *Escherichia coli*, we failed to find any clearly significant correlation between intracellular cyclic AMP concentration and degree of catabolite repression.(°)

2. Mutants have been isolated in our laboratory, which exhibit a completely normal pattern of catabolite repression which, however, turns out to be totally insensitive to the addition of cyclic AMP.

(°) - Recently Wayne and Rosen [4] found a decrease in cellular cyclic AMP concentration during transient repression in *Escherichia coli*. But they do not find a consistent correlation between permanent (catabolite) repression and intracellular cyclic AMP levels.

3. Also in *Escherichia coli*, CAP negative and cyclase negative mutants have been isolated which synthesize normal levels of galactokinase, while the formation of β-galactosidase is strongly repressed (5).

4. In *Klebsiella aerogenes*, it is observed that the synthesis of both proline oxidase and histidase are both quite independent of cyclic AMP under conditions of nitrogen starvation (6).

The sum of these observations rather strongly suggests the view that catabolite repression may be normally mediated, in many organisms and many if not most systems, by a complex of controls involving mediators other than, or in addition to, cyclic AMP itself.

Aknowledgements - I wish to thank Dr. Jean-Paul Aubert for many helpful suggestions and for the gift of the strain used in this work. I am grateful to Dr. Jacques Monod for stimulating discussions and criticism of the manuscript.

This work was supported by grants from the "Délégation Générale à la Recherche Scientifique et Technique", *the* "Centre National de la Recherche Scientifique" *and the* "National Institutes of Health".

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