ON THE CONTROL MECHANISM OF BACTERIAL GROWTH BY CYCLIC ADENOSINE 3', 5' - MONOPHOSPHATE

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SUMMARY

Inhibition of E.coli growth by cyclic adenosine monophosphate is observed in wild type strains cultured in glucose as carbon source, but not in a cyclic AMP receptor protein deficient mutant. A deletion mutant of the adenylate cyclase gene requires cyclic adenosine monophosphate for optimal growth. Using glucose as carbon source, 2 mM cyclic AMP promotes maximal rates of cell multiplication in this mutant; however higher concentrations of the nucleotide inhibit growth. Cell multiplication of wild type strains grown in glycerol is not affected by cyclic adenosine monophosphate. Nevertheless, in this carbon source the growth rate of the adenylate cyclase mutant is strongly inhibited by concentrations of this nucleotide beyond 0.1 mM. This suggests that growth inhibition by exogenous cyclic adenosine monophosphate is highly dependent on the intracellular levels of the nucleotide.

In a recent communication from this laboratory it was reported that the addition of cyclic adenosine 3', 5'-monophosphate (cyclic AMP) to the culture medium decreased the growth rate of the E.coli strain Hfr 3000 (1). The inhibition is specific since it was not observed with other nucleoside-phosphates and it occurred at concentrations of the cyclic nucleotide required for the reversal of permanent catabolite repression (2). In this sense, it is important to remark that inhibition of bacterial growth was observed only with those carbon sources, such as glucose or pyruvate, which determine catabolite repression (3, 4). The latter observation is interesting since it was evident that the addition of cyclic AMP to the cultures affected cell growth only when the intracellular concentration of the cyclic nucleotide was low enough to be limitant for β-galactosidase synthesis. Under conditions such as those imposed by growth in glycerol, malate or succinate, leading to the intracellular accumulation of the nucleotide (5-8) cyclic AMP did not affect bacterial growth (1).

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These results, and those of Buettner, Spitz and Rickenberg (8) showing some type of correlation between the rate of bacterial multiplication on different carbon sources and the intracellular concentration of cyclic AMP, could indicate that in the procariotic organisms there is a control mechanism mediated by the cyclic nucleotide which regulates cell growth rate. This phenomenom is strikingly similar to that previously found in mammalian cell cultures (9-11).

As a first approach to the characterization of this mechanism two possibilities should be analyzed. One of them is the implicance in this phenomenom of the cyclic AMP receptor protein (crp) which is required for the transcription of many catabolite repressible genes (12-15). The second possibility is that the inhibition observed might be the consequence of a block of some specific reaction leading to the metabolization of glucose and pyruvate. In addition, the paradox of an inhibitory effect on a wild type strain together with the growth stimulation found in adenylate cyclase deficient mutants of E.coli (16) must also be explained. This paper deals with these topics.

MATERIALS AND METHODS

The following bacterial strains were used throughout this work: PP6 (wild type; Hfr H, thi^-; also known as Hfr 1100) and PP47 (a nitroso guanidine mutant of PP6, cyclic AMP receptor protein deficient) were kindly provided by Dr. I. Pastan; CA 8000 (wild type; thi^-; Hfr H; also known as Hfr 3000) was a gift of Dr. J. Scaife; and CA 8306 (deletion of adenylate cyclase gene derived from CA 8000) was generously provided by Dr. J. Beckwith.

Bacterial cultures were grown in AG minimal medium (6) which contains 14g of K_2HPO_4, 6g of KH_2PO_4; 2g of $(NH_4)_2SO_4$, and 0.2g of $MgSO_4$ per liter supplemented with 5µg/ml thiamine and the additions indicated in each case. Other conditions were indicated in the preceding paper (1).

RESULTS AND DISCUSSION

Fig.1 shows the requirement of cyclic AMP receptor protein for the cyclic

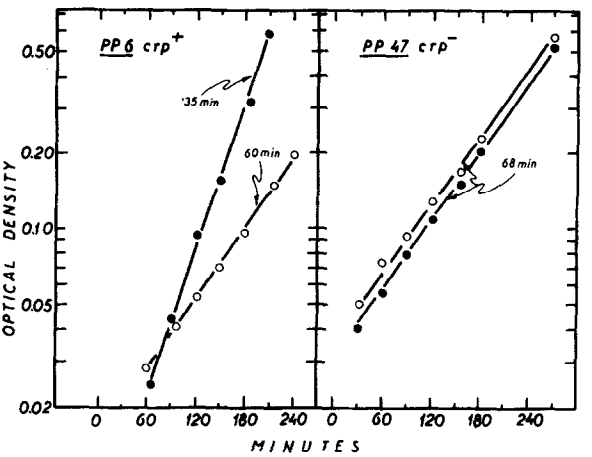

Fig. 1 Effect of cyclic AMP on the growth of PP6 (crp^+) and PP47 (crp^-) strains. The cells were grown in AG minimal medium, supplemented with 50 mM glucose, 0.1% vitamin-free casaminoacids and thiamine, containing (○) or not (●) 5 mM cyclic AMP. Conditions were as indicated under Methods.

nucleotide inhibition of cell growth. The mutant deficient in this protein grew at a lower rate than the parental strain and the nucleotide did not modify the growth rate. This result suggested that the control of bacterial multiplication by cyclic AMP could be exerted at the transcriptional level of some gene product, and not through a modulation of a preformed enzyme activity such as protein kinase as it occurs in eucariotic organisms (17).

It is well-known that using glucose as carbon source, cyclic AMP stimulates the growth of adenylate cyclase deficient mutants, when the nucleotide is added to the medium at concentrations of about 2 mM (16). In order to clarify the ambiguity of two opposite effects, stimulatory and inhibitory, on bacterial growth, a set of experiments were done with a deletion mutant of the adenylate cyclase gene (CA 8306; cya^-) isolated by J. Beckwith. No cyclic AMP was detectable in this strain (18) by the Gilman's procedure (19). As it is shown in Fig. 2A, when this strain was grown in a medium containing glucose and casaminoacids cell multiplication is stimulated by concentrations of cyclic AMP up to 2 mM. At higher concentrations of the nucleotide, growth was strikingly depressed. This result indicates that a certain intracellular concentration of cyclic AMP is required for maximal growth. Beyond this cyclic AMP level cell growth is inhibited.

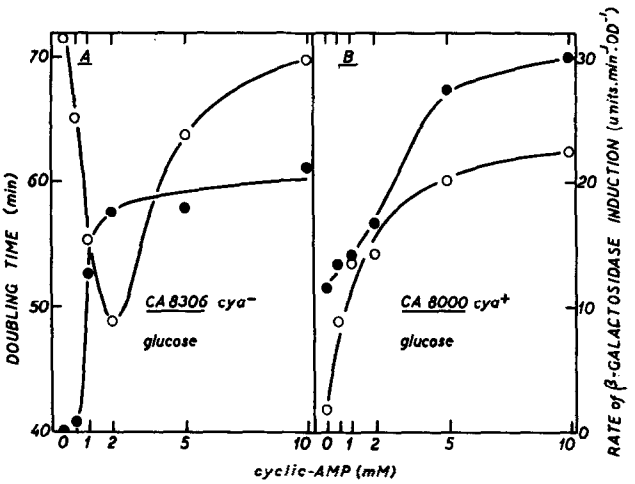

Fig. 2 Effect of cyclic AMP concentration on the doubling times (○) and the rate of β-galactosidase synthesis (●) after induction with 1 mM isopropyl β - D - thiogalactoside of CA 8306 (cya^-) and CA 8000 (cya^+) cultures. Cells were grown in AG minimal medium, supplemented with 50 mM glucose, 0.1% vitamin-free casaminoacids, thiamine and the indicated concentrations of cyclic AMP. Conditions were as those indicated under Methods.

The behavior of the parental strain is quite different. Optimum growth was observed in the absence of added cyclic AMP and the inhibition was maximal at concentrations of about 5 mM. These concentrations were also optimal for maximal rate of β-galactosidase induction. Under these conditions, the rate of induction increased more than 2-fold when compared with cultures not supplemented with the nucleotide (Fig. 2B). For reasons that we cannot explain at the moment, cyclic AMP requirements for optimal growth and β-galactosidase induction are coincident in the cya^- mutant but not in the parental wild type strain. However, the nucleotide levels found to be inhibitory in both strains are within the range of concentrations required for the relief of permanent catabolite repression (2), the diauxie lag (20), and for the increase in competence for bacterial transformation (21).

The cyclic AMP-promoted inhibition of growth rate observed when bacterial cultures are grown in glucose or pyruvate (1) could be due to a decrease in the activity of some enzymatic steps in the metabolic pathway of these sources, or in turn, to a more general phenomenom involving for example

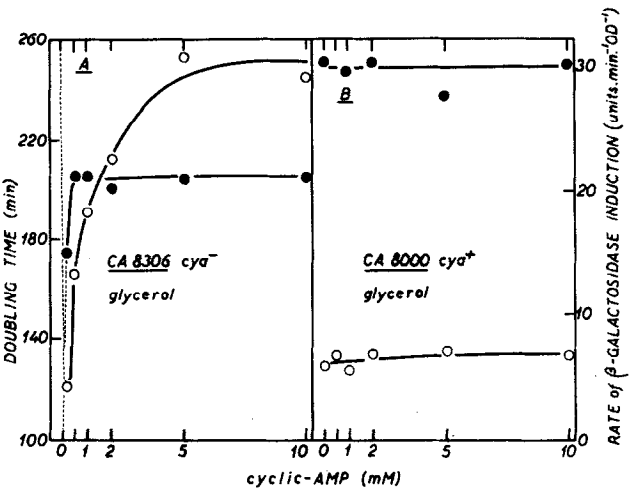

Fig. 3 Effect of cyclic AMP concentration on the doubling time (O) and the rate of β-galactosidase induction (●) of CA 8306 (cya⁻) and CA 8000 (cya⁺) cultures. Cells were grown in AG minimal medium supplemented with 68 mM glycerol, thiamine and the indicated concentrations of cyclic AMP. Conditions were as indicated under Methods.
The rates of growth and of β-galactosidase induction of CA 8306 (cya⁻) cultures carried out in the absence of cyclic AMP were negligible.

membrane transport, macromolecular synthesis (DNA, RNA, proteins), etc. The distinction between these two possibilities is relevant. The fact that no cyclic AMP effect was found in wild type E.coli cultured in glycerol, malate or succinate as carbon sources might favor the first possibility. However another possible explanation for this behavior would be that endogenous cyclic AMP levels in glycerol (or malate or succinate) cultured cells, were already high enough to sustain cyclic-AMP dependent functions even in the absence of exogenous added cyclic nucleotide. In fact, with glycerol as carbon source the intracellular cyclic AMP concentration is 3 to 10-fold higher than in glucose-cultured cells (5,8), and the rate of β-galactosidase induction is maximal (see Table I in reference 1 and Fig.3 in this paper). Adenylate cyclase deficient mutants are unable to grow in glycerol when the culture is not supplemented with cyclic AMP (16). In the case of CA 8306 (cya⁻) mutant, the nucleotide requirement is fulfilled by a low concentration of this compound (0.1 mM); under this condition the rate of β-galactosidase

induction is about 75 percent of the maximum (Fig. 3A). With higher concentrations of the cyclic nucleotide, doubling times increased to reach a maximum at 5 mM cyclic AMP. As expected, with the parental wild type strain growing in glycerol, no effect of the cyclic nucleotide was observed either at the level of β-galactosidase induction or at the level of cell growth. These results clearly show that the absence of cyclic-AMP effect on the growth of wild type strains cultured in certain carbon sources is the consequence of the relatively high levels of the nucleotide within the cells.

The nature of the mechanisms affecting growth, whose expression is under the control of cyclic-AMP remains unknown. A recent report (22) indicates that a progressive extracellular accumulation of methylglyoxal, stimulated by cyclic AMP, could be a factor promoting cell death in xylose-grown cultures. This does not seem to be the case in the phenomenom described in this paper, since cyclic AMP inhibition of cell growth was even observed at the beginning of the experiments, when the cell density was low (about 0.02 O.D.; $5x10^6$ cells/ml) and the slope of the semilogaritmic growth curves did not change in the period elapsed between four generations. On the other hand determinations of culture turbidity and cell viability were always parallel.

A great deal of work has been done in order to determine the mechanism of cyclic-AMP action on growth control in eucariotic cells. Some evidences indicate that the cyclic nucleotide could influence membrane transport of precursors (23-25). The possibility that a similar mechanism could regulate bacterial growth is under study.

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REFERENCES

1. Judewicz, N.D., De Robertis,Jr., E.M. and Torres, H.N. (1973) Biochem. Biophys.Res.Commun. 52, 1257-1262.

2. Perlman, R.L. de Crombrugghe, B. and Pastan, I. (1969) Nature 223, 810-812.
3. Perlman, R.L. and Pastan, I. (1968) Biochim.Biophys.Res.Commun. 30, 656-664.
4. Mc Fall, E. and Mandelstam, J. (1963) Biochem.J. 89, 391-398.
5. Pastan, I. and Perlman, R.L. (1970) Science 169, 339-344.
6. Makman, R.S. and Sutherland, E.W. (1965) J.Biol.Chem. 240, 1309-1314.
7. Peterkofsky, A. and Gazdar, C. (1971) Proc.Natl.Acad.Sci. U.S.A. 68, 2794-2798.
8. Buettner, M.J., Spitz, E. and Rickenberg, H.V. (1973) J. Bacteriol. 114 1068-1073.
9. Johnson, G.S., Friedman, R.M. and Pastan, I. (1971) Proc.Natl.Acad. Sci. U.S.A. 68, 425-429.
10. Sneppard, J.R., (1971) Proc.Natl.Acad.Sci. U.S.A. 68, 1316-1320.
11. Jiménez de Asua, L., Surian, S. Flawiá, M.M. and Torres, H.N. (1973) Proc.Natl.Acad.Sci. U.S.A. 70, 1388-1392.
12. Emmer, M., de Crombrugghe, B., Pastan, I. and Perlman, R.L. (1970) Proc.Acad.Sci. U.S.A. 66, 480-487.
13. Riggs, A.D., Reiness, G. and Zubay, G. (1971) Proc.Natl.Acad.Sci. U.S.A. 68, 1222-1225.
14. Schwartz, D. and Beckwith, J., in "The lac Operon" D. Zipser and J. Beckwith, eds. (1970) Cold Spring Harbor Laboratory of Quantitative Biology, N.Y., 417-422.
15. Nissley, P., Anderson, W.G., Gallo, M., Pastan, I. and Perlman, R.I. (1972) J.Biol.Chem. 247, 4264-4269.
16. Perlman, R.L. and Pastan, I. (1969) Biochem.Biophys.Res.Commun. 37, 151-154.
17. Jost, J.P. and Rickenberg, H.V. (1971) Ann.Rev.Biochem. 40, 741.
18. Beckwith, J., Personal communication.
19. Gilman, A.G. (1970) Proc.Natl.Acad.Sci. U.S.A. 67, 305-312.
20. Ullmann, A. and Monod, J. (1968) FEBS Letters 2, 57-60.
21. Wise,Jr., E.M., Alexander, S.D. and Powers, M. (1973) Proc.Natl.Acad. Sci. U.S.A., 70, 471
22. Ackerman, R.S., Cozzarelli, N. R. and Epstein, W., J.Bacteriol., in press.
23. Kram, R., Mamont, P. and Tomkins, G.M. (1973) Proc.Natl.Acad.Sci. U.S.A., 70, 1432-1436.
24. Hauschka, P.V., Everhart, C.P. and Rubin, R.W. (1972) Proc.Natl.Acad. Sci., U.S.A., 69, 3542-3546.
25. Grimes, W. J. and Schroeder, J.L. (1973) J. Cell Biol. 56, 487-491.

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