THE EFFECT OF CYCLIC AMP ON ANAEROBIC GROWTH OF ESCHERICHIA COLI

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SUMMARY

Adenosine 3',5'-cyclic phosphate (cyclic AMP) stimulated a cyclic AMP-deficient mutant strain of Escherichia coli to grow anaerobically on glucose in a minimal medium and in media supplemented with nitrate or casein hydrolysate. Cyclic AMP was found to stimulate the production of the formic hydrogenlyase system in this mutant strain, but had no effect on its ability to carry out anaerobic reductions of nitrate or nitrite. It was also observed that CO_2 stimulated the anaerobic growth of the mutant in the absence of cyclic AMP.

INTRODUCTION

All of the studies reported thus far dealing with the growth of cyclic AMP-requiring mutants of E. coli have employed aerobic conditions of growth. There is no report in the literature concerning the growth of these strains under strictly anaerobic conditions. In the studies described in this report, we have attempted to ascertain some of the effects cyclic AMP has on the anaerobic metabolism of glucose by a cyclic AMP-deficient strain of E. coli.

MATERIALS AND METHODS

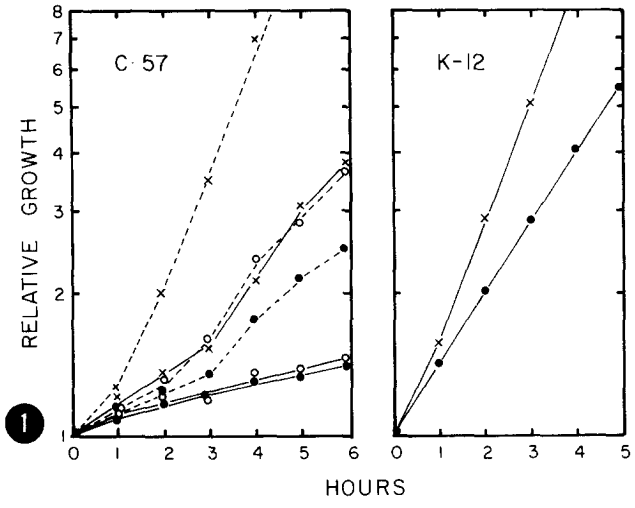
The bacterial strains used in these studies were E. coli K-12-701 (White, 1968), and a cyclic AMP-requiring mutant derived from this parent using the technique of Perlman and Pastan (1969). The mutant was designated strain C-57 and cannot synthesize cyclic AMP as determined using the Schwarz/Mann cyclic AMP radioimmunoassay kit and the assay of Wastila et al. (1971). Stock cultures of the mutant were prepared and stored at 2 C on plates of the isolation medium supplemented with 0.5 mM cyclic AMP. Fresh plates were prepared every 3-4 weeks and checked for retention of the mutant characteristics.

The minimal basal medium used for growing these cells has been described elsewhere (Okinaka and Dobrogosz, 1967). A 0.02 M concentration of glucose was added to the medium prior to inoculation. In some experiments this medium was supplemented with either 3.6 mM nitrate, 3.6 mM nitrite or 0.25% casein hydrolysate. Anaerobic conditions were produced and maintained, and cell growth determined turbidimetrically as described elsewhere (Dobrogosz, 1965). Plate cultures were incubated in anaerobic jars that had been evacuated and flushed three times with an atmosphere of 100% N_2 or 95% N_2-5% CO_2. When desired, absorption of metabolically-generated CO_2 was effected by placing plates containing 20% KOH and large filter paper wicks in these jars.

The appearance and utilization of nitrate in the culture fluid was measured as previously described (Dobrogosz, 1965). Hydrogen formation was measured under anaerobic conditions using the manometric procedure described by Umbreit et al. (1964). Cells grown under anaerobic conditions in tightly-sealed screw cap tubes were harvested at hourly intervals washed with 0.05 M sodium phosphate buffer (pH 7.3), and placed in Warburg respirometer vessels containing 0.20 ml of 20% KOH in the center well and 0.5 ml of 0.1 M sodium formate in the side arm. Hydrogen evolution was followed for 60 min after mixing of cells and formate. The specific activity of this formic hydrogenlyase system was defined as the μmoles of H_2 evolved/hr/mg cell dry weight. Growth of anaerobic colonies on plates was measured using an Ehreinreich Photo-Optical Profile Projector and expressing the results as colony diameter in mm.

RESULTS

The data presented in Figure 1 show the effect of cyclic AMP on anaerobic growth of strain C-57 in media containing glucose, glucose plus nitrate or glucose plus casein hydrolysate. Growth of the K-12 strain on glucose and glucose plus casein hydrolysate is also shown for comparative purposes. It can be seen that growth of the mutant strain in the absence of cyclic AMP was poor in the glucose medium and in the nitrate supplemented medium. The addition of cyclic AMP stimulated the growth under these conditions with the stimulation



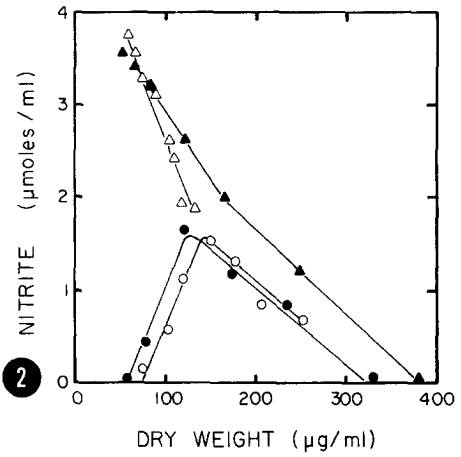


Figure 1. Effect of cyclic AMP on anaerobic growth of E. coli. The solid lines indicate relative growth observed in the absence of added cyclic AMP while the dashed lines indicate growth observed in the presence of 1.25 mM cyclic AMP. ●, 0.02 M glucose; O, 0.02 M glucose plus 3.6 mM KNO_3; x, 0.02 M glucose plus 0.25% casein hydrolysate.

Figure 2. Effect of cyclic AMP on differential rates of anaerobic nitrate and nitrite reduction by E. coli. All the cultures contained 0.02 M glucose plus 0.25% casein hydrolysate in addition to: O—O, 3.6 mM KNO_3; ●—●, 3.6 mM KNO_3 plus 1.25 mM cyclic AMP: Δ—Δ, 3.6 mM KNO_2; ▲—▲, 3.6 mM KNO_2 plus 1.25 mM cyclic AMP.

more pronounced when nitrate was also present. The mutant was able to grow reasonably well in the casein hydrolysate supplemented medium even without added cyclic AMP although the addition of this nucleotide stimulated this growth to the wild-type level.

There is no data available at the present time to explain how cyclic AMP is able to stimulate anaerobic growth in the minimal glucose medium. It was considered possible that stimulation of growth by cyclic AMP in the nitrate containing medium might be caused by an effect of this nucleotide on the nitrate or nitrite reducing abilities of these cells. The experiments presented in Figure 2 indicated that this was not the case. The C-57 cells were grown anaerobically with and without cyclic AMP in glucose media containing 3.6 mM nitrate or 3.6 mM nitrite. Samples were taken to measure growth and

Table 1. The effect of cyclic AMP on development of the anaerobic formic hydrogenlyase system of E. coli.

Time of Culture Growth (Hr)	Formic Hydrogenlyase Specific Activity[a]	
	Minus Cyclic AMP	Plus Cyclic AMP
0	0.0	0.0
1	1.4	2.6
2	1.0	13.5
3	0.9	13.4
4	2.0	10.9
5	5.0	10.1

[a] μmoles H_2 formed/hr/mg cell dry weight

the nitrite content of the culture fluids, and the data were plotted on a differential basis. It can be seen that cyclic AMP had no appreciable effect on the ability of these cells to reduce either of these electron acceptors.

The data presented in Table 1 showed that cyclic AMP had a pronounced effect on the ability of strain C-57 to develop its anaerobic formic hydrogenlyase system. The cells were grown in the casein hydrolysate supplemented medium. It can be seen from these data that whereas the cells grown in the absence of cyclic AMP were unable to produce appreciable amounts of H_2, the cyclic AMP-supplemented cells developed an active formic hydrogenlyase system.

During the course of comparable experiments in which attempts were made to grow the mutant cells in the Warburg vessels, it was observed that the cultures grew very poorly or not at all in the absence of cyclic AMP in those vessels containing KOH as compared to identical cultures grown in the anaerobic tubes. The parental strain grew equally well in the KOH-containing vessels or in the culture tubes. These observations indicated that the C-57 cells had a requirement for CO_2 even in the rich glucose-casein hydrolysate medium when grown in the absence of cyclic AMP.

This requirement for CO_2 when the mutant cells were grown in the absence

Table 2. The effect of cyclic AMP and CO_2 on colony growth of E. coli.

Atmospheric Conditions	Cyclic AMP	Colony Size (mm) Strain C-57	Strain K-12
Anaerobic	None	0.9 ± 0.06	1.3 ± 0.01
+ CO_2	1.25 mM	1.8 ± 0.09	1.4 ± 0.08
Anaerobic	None	0.4 ± 0.09	0.9 ± 0.02
-CO_2	1.25 mM	1.3 ± 0.02	0.8 ± 0.02

of cyclic AMP was further substantiated using colony size as a criterion of growth rather than cell turbidity. The results presented in Table 2 showed that the mutant colonies grown without CO_2 and cyclic AMP were considerably smaller than those grown without the cyclic AMP but in the presence of CO_2. When cyclic AMP was present the colony sizes were comparable to those of the parental strain. Although the size of the K-12 colonies was not affected by cyclic AMP, there was a reduction in size of the K-12 colonies which had been grown in the absence of CO_2.

DISCUSSION

Recent studies in this laboratory have shown that the effect of cyclic AMP on the growth and metabolism of E. coli is not limited to its role in the catabolite repression phenomenon. These studies conducted primarily on the C-57 mutant strain have demonstrated that cyclic AMP participates in 1) controlling the level of the organism's cytochrome components (Broman and Dobrogosz, 1972), 2) determining their phospholipid composition (Goldenbaum, Edwards and Dobrogosz, 1973), 3) the functioning of their oxidative phosphorylation system (Keyser et al. 1973), 4) determining the structural relationship of the cell's surface components as visualized by electron microscopy (Weeks and Dobrogosz, 1973), 5) controlling the transport of metabolites such as glucose-6-phosphate (Ezzell and Dobrogosz, 1972), and 6) determining the sensitivity of these cells to growth in high ionic environments (Dobrogosz, unpublished data).

A characteristic that all of these cyclic AMP-influenced systems appear to have in common is that they are physically and functionally associated with the organism's membrane systems. It is postulated, therefore, that cyclic AMP may play a key role in regulating certain membrane functions in E. coli. The findings described in the present study seem to be consistent with this proposed role for cyclic AMP. The formic hydrogenlyase system is known to contain an anaerobic cytochrome component and the hydrogenase portion of this system is known to be associated with the membrane (Gray and Gest, 1965). Whether or not the anaerobic CO_2 requirement observed with the cyclic AMP-deficient cells is also a membrane associated reaction is not known. It would appear that the membrane system formed during either aerobic or anaerobic growth of E. coli is subject to some regulatory control by cyclic AMP. Clearly, however, more work along this line is called for before this postulated role for cyclic AMP can be more clearly defined.

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