RELEASE OF GLUCOSE REPRESSION OF OXIDATIVE PHOSPHORYLATION IN ESCHERICHIA COLI B BY CYCLIC ADENOSINE 3′,5′- MONOPHOSPHATE

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Summary

Growth of Escherichia coli B in media containing glucose and 2.5 - 5 mM cyclic adenosine 3′,5′-monophosphate (cAMP) yields cells capable of full oxidative phosphorylation while growth in media containing glucose without cAMP produces cells capable of less than 10% of full phosphorylation efficiency. Lower concentrations of cAMP result in cells capable of intermediate efficiency. The rate of growth in glucose-cAMP cultures is less than in glucose medium and the rate of respiration of cells obtained from glucose-cAMP cultures is less than that of controls without cAMP in proportion to the amount of cAMP present. Unlike the respiration of cells derived from non-repressive media the respiration of glucose-cAMP cells is inhibited by 2,4-dibromophenol.

We have described the repression of oxidative phosphorylation in Escherichia coli B by growth in media containing glucose, and have shown that exhaustion of glucose in the presence of other substrates restored full oxidative phosphorylation[1]. The cyclic mononucleotide adenosine 3′,5′-monophosphate (cAMP) has been implicated in the control of synthesis of enzymes subject to catabolite repression[2,3,4]. It was therefore of interest to compare organisms grown in the presence of glucose and cAMP with those previously described in order to test the sensitivity to cAMP of control of synthesis of the apparatus of oxidative phosphorylation. The results show that glucose repression of oxidative phosphorylation is released by cAMP but that concomitantly respiration dependent upon glucose is inhibited and is further diminished by the uncoupler 2,4-dibromophenol.

Materials and Methods

Escherichia coli B was cultivated in 400 ml of medium contained in 2.8

L Fernbach flasks. The composition of growth media, the conditions of growth and the methods of harvesting and washing have been described[1]. The term "minimal medium" indicates that amino acids were not added.

Oxidative phosphorylation was assayed and respiration was measured as given previously[1]. Protein was estimated by the biuret method, using egg white lysozyme as standard.

Cyclic adenosine 3′,5′-monophosphate was purchased from the Sigma Chemical Co. and was added to culture media just before inoculation.

Results

As shown in Table 1 Escherichia coli B, grown in glucose-minimal medium in the presence of 2.5 mM cAMP, is capable of full oxidative phosphorylation (P/2e- = 3.3). Organisms grown in unsupplemented glucose-minimal medium or in the same medium containing 2.5 mM 5′-AMP phosphorylate much less efficiently (P/2e- = 0.2). Cells grown in the presence of cAMP esterify phosphate more extensively during the 15 min. anaerobic preincubation period than do

Table 1. Effect of adenosine monophosphates on oxidative phosphorylation in E. coli B grown in glucose-minimal medium.

Addition to growth medium	Pi esterified[a] before and after O_2				Pyridine nucleotides[a] before and after O_2				Δ Pi	Δ PNH	App. P/2e⁻
			+ DBP[b]		NADH		NADPH				
	0[c]	5 sec	0[c]	5 sec	0	5 sec	0	5 sec			
None	4.23	4.62	2.05	2.20	1.26	0.30	0.48	0.37	0.24	1.02	0.24
5′-AMP 2.5 mM	3.33	3.90	1.55	1.89	1.07	0.09	-	-	0.23	0.98	0.23
cAMP 2.5 mM	6.45	8.33	3.00	3.18	0.73	0.28	0.28	0.23	1.69	0.51	3.31

[a] μmoles/g protein.

[b] 250 μM 2,4-dibromophenol added before Tris-P_i^{32} addition.

[c] Pi esterified during 15 min anaerobic preincubation.

control cells or cells grown in the presence of 5′-AMP, a characteristic of bacteria capable of full oxidative phosphorylation[1]. Only half as much reduced pyridine nucleotide is oxidized in cAMP cells following oxygen addition as compared with control or 5′-AMP cells. This inequality cannot alone be the cause of the larges increase of apparent P/2e- since cAMP cells esterify more than seven times as much phosphate during oxygenation as do control cells. Furthermore, nearly 90% of oxygen-dependent phosphate esterification is abolished by DBP in cAMP cells.

Preincubation of washed, glucose-grown cells for 30 min. in the presence of 5 mM cAMP immediately before assay of oxidative phosphorylation had no effect upon anaerobic phosphorylation or the small amount of oxygen-dependent phosphate esterification.

The rate of growth of E. coli B in the presence of cAMP and glucose is slower than that in the presence of glucose alone, an observation that has been made by others[3]. A simple relation between growth rate and cAMP concentration has not been observed by us, however. For example, the rate of growth in glucose-minimal medium is about 1.3 doublings/hr for more than 90% of the time course of growth (up to O.D. = 1 at 650 nm). Yet the addition of 2.5 mM cAMP at the time of inoculation slows the rate of growth to 1 doubling/hr during the first third of the growth period and further to 0.6 doubling/hr during the remainder. 2.5 mM 5′-AMP and 5′-ATP are without effect upon the growth rate in glucose-minimal medium.

In order to study the comparative effects of cAMP on growth rate, oxidative phosphorylation and respiration, glucose-Casamino Acids medium (glucose-CAA) was used, since in preliminary experiments it was found that growth of E. coli B in glucose-CAA medium containing cAMP was slowed as compared with growth in unsupplemented glucose-CAA medium, but that the rate of growth did not change with time. The results obtained by varying cAMP concentration in glucose-CAA medium are shown in Figure 1. Saturation of the effect of cAMP on oxidative phosphorylation occurs at about 2.5mM cAMP but

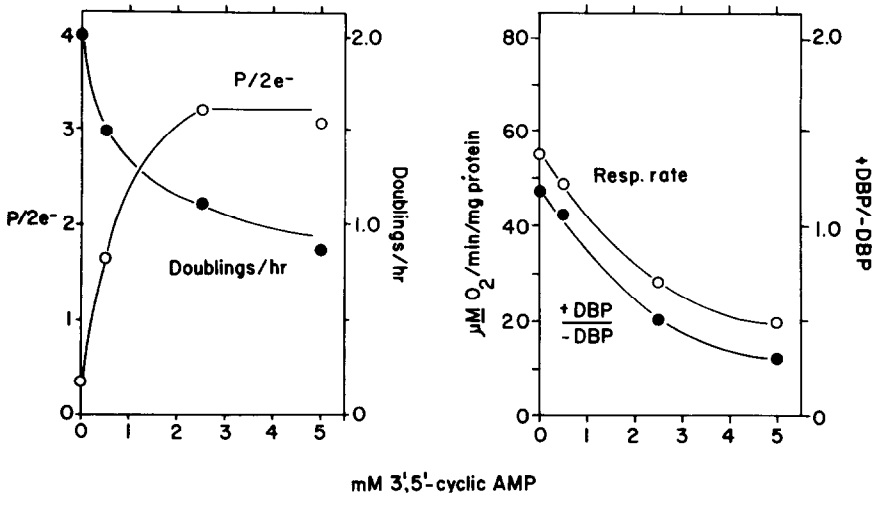

Figure 1. Effect of variation of 3',5'- cyclic AMP upon oxidative phosphorylation, growth rate and respiration of glucose grown E. coli B. +DBP/-DBP: ratio of O_2 consumption rate in presence of 140 μM 2,4-DBP to that in its absence (glucose substrate).

growth rate is lessened further by increasing cAMP to 5 mM. The rate of oxygen consumption during glucose oxidation by resting cells declines with increasing cAMP and furthermore 2,4-dibromophenol, rather than increasing the respiration rate (e.g. as in the case of lactate-grown cells with full oxidative phosphorylation[1]), inhibits oxygen utilization in a manner proportional to the concentration of cAMP in the growth medium. Lower concentrationsof DBP inhibit respiration less, but at no level of DBP is respiration stimulated (50 - 150 μM DBP).

To test the effect upon growth and respiration in a medium normally permitting the growth of cells capable of full oxidative phosphorylation, those parameters were measured with cells derived from lactate-minimal and lactate-CAA media, supplemented or unsupplemented with 2.5 mM cAMP. No significant differences were detected between cells from either medium with re-

gard to the rates of growth or respiration in the presence of glucose or lactate. DBP stimulated respiration as reported earlier[1] even when cells were used which had been grown in the presence of cAMP.

Discussion

In the present study half-maximal effects of cAMP upon all parameters measured fell within the range 1 - 2 mM cAMP, an insufficiently narrow range to suggest that a single event (such as stimulation of transcription) is directly responsible for all the changes observed. The concentration of cAMP necessary for a maximal effect upon the efficiency of oxidative phosphorylation (2.5 - 5 mM) is similar to the amount of cAMP releasing glucose repression of synthesis of tryptophanase and other enzymes in other strains of *E. coli*[5].

The observation that growth in glucose-cAMP medium results in slowing of respiration of resting cells differs from the findings of Perlman and Pastan[6] and of Moses and Sharp[7]. Both groups reported slight stimulation of CO_2 release from glucose in growing *E. coli*, and Moses and Sharp presented evidence that glucose flux through the Embden-Meyerhof-Parnas pathway of glycolysis was augmented in the presence of cAMP. Their data, however, conflict with those of Perlman and Pastan, who showed that $^{14}CO_2$ production from glucose-1-^{14}C *increased* in the presence of cAMP. No information about the availability of oxygen was provided by Perlman and Pastan; Moses and Sharp suggested that at high population densities their cultures probably were oxygen limited. Oxygen consumption has apparently not been directly measured by others.

The similarity of dependence on the concentration of cAMP of the decrease of respiration rate and the inhibition of respiration by DBP suggests that the two effects share a common cause, and we suggest that the effect is centered within the cell membrane. Massive changes of the protein content of cAMP-treated bacteria have been described[7], and it may be that a change of membrane properties is a consequence of the derepressed synthesis

of proteins associated with respiration and oxidative phosphorylation. If it is assumed that the principal effect of DBP is to increase proton permeability through the cell membrane[8], then it might be anticipated that the interaction of proton transfer across the membrane and linkage of such transfer to ATP synthesis is altered in glucose-cAMP cells. It was previously shown that stimulation and respiration by DBP occurred only in cells capable of full oxidative phosphorylation[1], yet the opposite effect occurs with glucose-cAMP cells. It does not appear that respiratory stimulation by an uncoupler is a necessary characteristic of full oxidative phosphorylation. A direct effect of cAMP upon the membrane is unlikely since cAMP, incubated with glucose-grown cells, does not bring about an increase in the apparent P/2e- value.

The diminution of growth rate brought about by cAMP is also unexplained, nor has it been explained by other investigators. It is possible that the intracellular phosphate potential or the "energy charge" may exceed allowable values in glucose-cAMP cells, resulting in a general slowing of biosynthesis. However, it remains to be seen if oxidative phosphorylation is operative in growing cells in the presence of glucose and cAMP.

The results reported here indicate that at least part of the apparatus of oxidative phosphorylation is under control similar to that of other catabolic enzymes. However, restoration of the property of stimulation of respiration by the uncoupler 2,4-dibromophenol does not accompany the acquisition of full oxidative phosphorylation. Hence, a complete reversal of glucose repression of oxidative phosphorylation and related reactions does not occur due to the addition of cAMP.

Acknowledgements

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