RELATIVE DEPENDENCE ON INTRACELLULAR CYCLIC ADENOSINE-3′,5′-MONOPHOSPHATE LEVELS OF CATABOLITE REPRESSIBLE PROTEINS IN *ESCHERICHIA COLI*

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1. Introduction

Much work has been devoted to the study of the mechanism of catabolite repression in *E. coli* [1–3]. By the use of very sophisticated techniques, it has been possible to reconstitute in vitro systems for the synthesis of catabolite repressible enzymes [4–6]. There is some evidence that various operons differ in their sensitivity to catabolite repression [7]. The kinetics of bulk protein synthesis observed upon an increase of endogenous cyclic adenosine-3′,5′-monophosphate (cyclic-AMP) will reflect the derepression of all the induced catabolically repressible proteins. Thus, if the majority of catabolite repressible operons show similar sensitivity to this type of repression, one could expect to see an increase in protein synthesis that is proportional to the increase in cyclic-AMP. But if different operons vary widely in their sensitivity, a non-proportional increase in protein synthesis is expected.

2. Materials and methods

P4 X 8 ts 84, a derivative of P4 X 8, was a generous gift of Dr Y. Hirota. The cultures were incubated in medium 63 [8] supplemented with 0.5% casamino acids. Glycerol 0.4% was used as a carbon source. Protein synthesis was followed by incorporation of [^{3}H]leucine into trichloracetic acid precipitates as previously described [9]. Cell numbers were counted in a Coulter counter model F (Coultronic France). Samples were diluted a thousand times in a buffer 0.1 M sodium phosphate pH7, containing 1 g/l sodium citrate, 0.4% formaldehyde, filtered through a millipore filter (0.1 μm). For cyclic AMP assay, samples (4.5 ml) of the culture were filtered rapidly through membrane filters (Millipore HA 0.45 μm). The filters were suspended immediately without washing in 1 ml of 1 N hot perchloric acid and kept for 10 min at 100°C. For assaying cyclic-AMP, the heated extract was homogenized with a micro-potter and centrifuged. The supernatant was collected, neutralized with potassium carbonate and then appropriate dilutions were used for the assay. A highly sensitive radioimmunoassay of cyclic AMP was employed. In this new assay, reported by H. Cailla et al [10], cyclic AMP is first converted into 2–0′ succinyl cyclic-AMP. Like an earlier assay [11] this one is based on the competition between iodinated and cold antigen for the binding site of an antibody directed against succinyl cyclic-AMP. The data obtained from the auto gamma scintillation counter on a perforated tape, were analyzed by a computer. Duplicate assays differed rarely by more than 5%. Calculation of intracellular concentrations of cyclic-AMP in units of molarity is based on an accessible volume of 7.5×10^{-13} ml/bacterium.

β-galactosidase synthesis was induced by addition to the culture medium of isopropyl β-D-thio galactoside (IPTG) β-galactosidase activity was assayed as outlined by A. Ullmann et al. [12]. One unit of β-galactosidase catalyzes the hydrolysis of 1 nmole of orthonitrophenyl β-D-galactoside per minute at 28°C pH 7.

3. Results and discussion

For this study we had to find two essential conditions: 1) a medium that causes a low intracellular cyclic AMP concentration and 2) a medium that causes a slow increase of this level. We found that addition of 0.5% casamino-acids to the minimal medium used, fitted perfectly those conditions. This is shown in fig. 1A. The endogenous molarity of cyclic AMP can be evaluated since we monitored the number of cells per milliliter of culture. Using, 7.5×10^{-13} ml per bacterium, as the accessible volume, one can calculate that this molarity varies from 0.8×10^{-5} M to 3.8 $\times 10^{-5}$ M during the growth. These values are in very good agreement with those reported by Buettner et al. [13] for the conditions of catabolite repression and derepression. Under our conditions the amount of β-galactosidase synthesized reflects exactly the increase in cyclic-AMP level (fig. 1B). Using minimal medium supplemented with 10 mM glucose or 20 mM glycerol, these authors obtained values of 1.2×10^{-5} M and 4.3×10^{-5} M respectively.

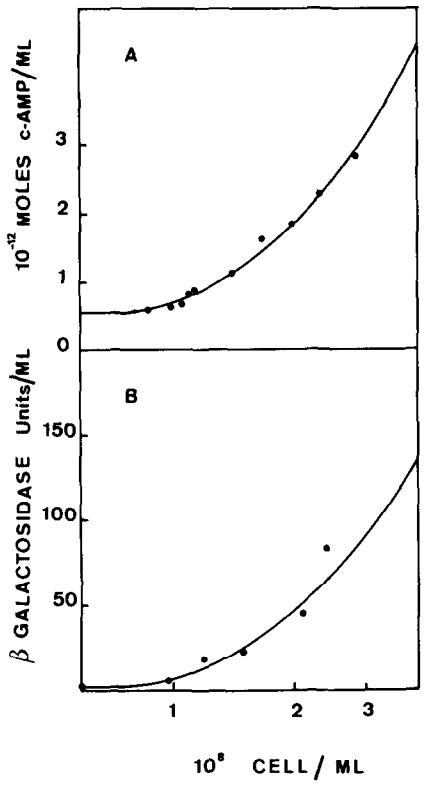

Fig. 1. Increase of intracellular cyclic-AMP concentration per ml of culture during growth. Aliquots were removed from the culture at intervals and: (A) cyclic-AMP was assayed as described in Materials and methods; (B) the number of units of β-galactosidase per ml of culture was determined.

During logarithmic growth, protein synthesis can be considered as a steady state mechanism. Consequently, the protein content per cell should be constant throughout this phase. However, since, in the presence of casamino acids, the intracellular cyclic-AMP level increases, a similar increase in protein content per cell is expected. The intracellular concentration of the cyclic nucleotide is mainly regulated by adenyl cyclase activity and by the rate of cyclic-AMP exit from the cell [13]. The pattern of the increase of cyclic-AMP level is very peculiar in that it is stepwise (fig. 2). Two plateaus are first observed, then, when all the catabolites have been used up a continuous increase occurs. The first part of the curve is probably due to the sequential utilization of the catabolites, responsible for repression, and might reflect a stepwise activation of adenyl cyclase since a decrease of the rate of cyclic-AMP exit from the cell is not very likely to occur. The protein content of the cells during growth follows exactly the nucleotide increase (fig. 2).

This parallel increase of protein synthesis is significant since the two parameters proteins and cyclic-AMP were independently assayed in two different aliquots of a same sample of the culture. It just means that the sensitivity to catabolite repression of the majority of catabolite repressible proteins is of the same order. This is better shown on Fig. 3. The mid-point of the plot of the percentage of β-galactosidase derepression vs. cell number per milliliter of culture, is practically coincident with the transition point for bulk protein synthesis. This point occurs at a density of 2×10^8 cells/ml which corresponds to an intracellular concentration of 2.6×10^{-5} M cyclic-AMP. In other words our result suggests that most of the catabolite repressible proteins share with β-galactosidase the same dependence on cyclic-AMP concentration. However, it is obvious that discrete differences in this respect like the one which exists between the operons lactose and arabinose [7] escape detection by this method.

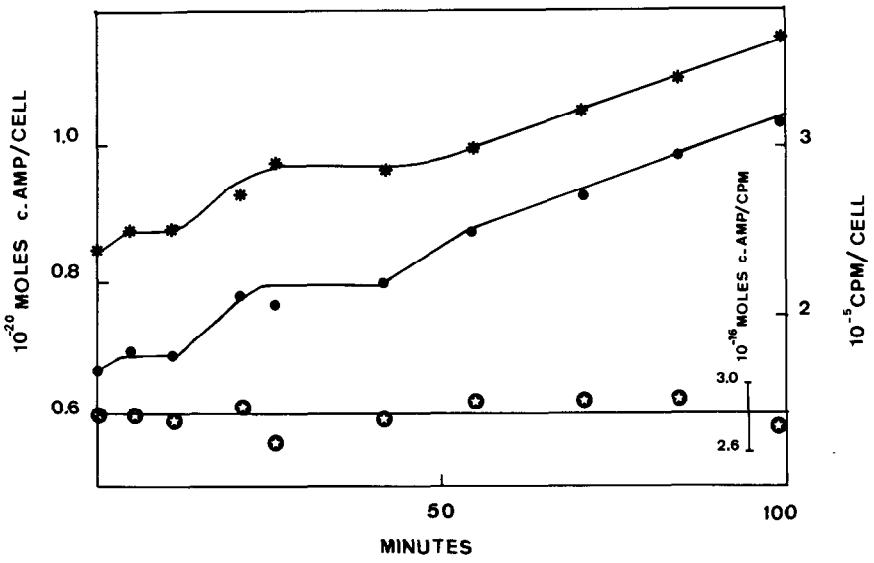

Fig. 2. Parallel increase in cyclic-AMP and protein content per cell during growth. Aliquots were removed from the culture at intervals and intracellular concentration of cyclic AMP- (☆) as well as protein content per cell (i.e. CPM/cell) (*) were determined. The ratio of cyclic-AMP to protein content per cell is shown at the bottom of the figure (●).

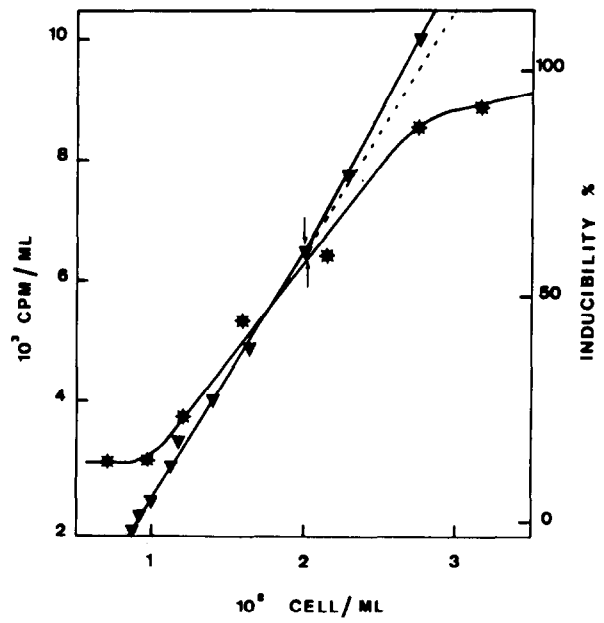

Fig. 3. Increase in protein content per ml of culture (▼) and per cent of β-galactosidase induction (★) during growth.

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