ON THE SPECIFICITY OF CYCLIC AMP ACTION IN *ESCHERICHIA COLI*

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1. Introduction

The role of adenosine 3′,5′-cyclic monophosphate (cAMP) in bacteria is fairly well understood. Both *in vivo* and *in vitro* experiments have shown that the nucleotide is essential for a correct expression of bacetrial genes sensitive to catabolite repression [1]. The mechanism of the regulatory role of cAMP has been studied *in vitro*. It was shown that glucose and other compounds bringing about the catabolite repression were causing a decrease in the cAMP concentration in cells [2]. Exogenous cAMP, when supplied in a sufficient concentration to the repressed culture, controls positively the initiation of transcription of the genes sensitive to the catabolite repression [3]. Moreover, there are certain indications that cAMP affects the translation of the genes as well [4, 5]. Although such effects are well demonstrated, there is not much known about their specificity. For this reason, we asked whether cAMP may be replaced by its analogues (fig. 1) in its effect on the catabolite repression.

2. Experimental

8-Bromo adenosine 3′,5′-cyclic monophosphate, 2′-*O*-methyl adenosine 3′,5′-cyclic monophosphate and 2-amino purine riboside 3′,5′-cyclic monophosphate, synthesized by Smrž and Farkaš, were used in this study.

The lactose operon, a well defined inducible system sensitive to the catabolite repression, was used. *Escherichia coli* B were grown on a mineral medium supplemented with glycerol [6]. The cells were sensitized by the EDTA treatment according to Perlman and Pastan [7]. The lac operon was induced by adding isopropyl 1-thio-β-D-galactopyranoside (Lachema, Czechoslovakia) in a concentration of 5×10^{-4}M. The induced culture was incubated for 30 min at 37° on a Dubnoff shaker and then divided into pre-warmed Erlenmeyer flasks with the aliquots of the tested compounds. Glucose was added in a concentration of 10^{-2} M, cAMP and its analogues were added in concentrations of 10^{-3} M. The cultures were incubated and aliquots (0.5 ml) were withdrawn, chilled in ice

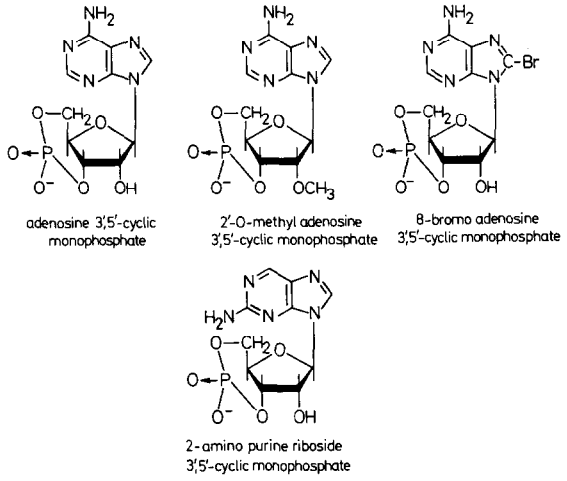

Fig. 1.

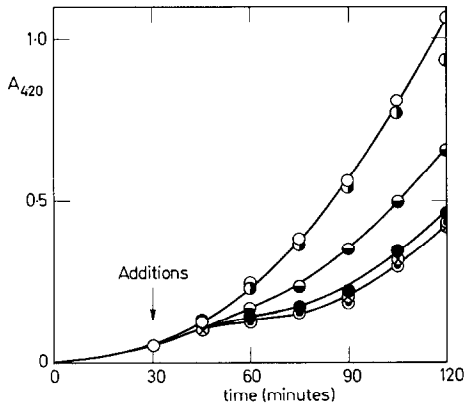

Fig. 2. The effect of cAMP and its analogues on the catabolite repression of the β-galactosidase induction in *E. coli*. Glucose was added to the culture 30 min after the induction (⊕). cAMP (◑), 2′-*O*-methyl adenosine 3′,5′-cyclic monophoaphate (◒), 2-amino purine riboside 3′,5′-cyclic monophosphate (●) and 8-bromo adenosine 3′,5′-cyclic monophosphate (⊗) were added to the aliquots of the culture. The control (○) is without glucose.

and mixed with a drop of toluene and 100 μl of a deoxycholate solution (100 μg/ml). The induced β-galactosidase was determined spectrophotometrically with *o*-nitrophenyl β-D-galactopyranoside (Lachema, Czechoslovakia) as substrate [8].

3. Results and discussion

The results show (fig. 2) that the effect of cAMP on the catabolite repression is very specific. The analogues derived from cAMP by the base modifications are not active. The methylation of the sugar moiety of cAMP does not destroy the activity of the nucleotide completely. However, the activity of 2′-*O*-methyl adenosine cyclic phosphate is lower than that of cAMP. Moreover, the specificity is evident from the inability of any of the tested analogues to compete with the cAMP action. The results are consistent with the findings on the high specificity of cAMP action during the transcription *in vitro* in a bacterial system [3].

The opposite tendency is observed in animal systems [9]. Certain changes in the base structure do not interfere with the cAMP activity during hormonal regulation. Some analogues of this type penetrate the cell membrane more easily and exert stronger effects than observed with the unmodified cAMP. Modifications in the ribose moiety and phosphate bond, unlike the base modifications, reduce the activity of cAMP.

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