CYCLIC ADENOSINE-3′, 5′-MONOPHOSPHATE AND THE INHIBITION OF RIBONUCLEIC ACID SYNTHESIS BY PROFLAVINE

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1. Introduction

Proflavine belongs to a group of chemically synthesized drugs, the acridines, which binds nucleic acids and has been used as a biochemical tool to study the properties of DNA and RNA *in vivo* and *in vitro* [1]. The binding of proflavine to the bacterial chromosome results in a rapid inhibition of the DNA dependent RNA synthesis and has therefore provided a means to study enzyme synthesis in the absence of transcription [2]. During the course of some experiments on the catabolite repression of the enzyme tryptophanase we have found that the cyclic nucleotide adenosine-3′, 5′-monophosphate (cAMP) hinders the uptake of proflavine by *Escherichia coli* during growth. We have also found that the overall synthesis of RNA and protein are inhibited by proflavine to a lesser degree when cAMP is present. The significance of our findings is apparent since proflavine together with cAMP has been used in earlier studies [3–5] in discerning the role of cAMP in the synthesis of catabolite repression sensitive enzymes.

2. Experimental

The strain used in this work, *Escherichia coli* RS1, and the culture medium have been described previously [6]. Cells in the exponential phase, grown on glycerol at 37°, were used in all the experiments (about 3.5×10^8 cells/ml). Absorbance was measured with a Gilford model 2400 spectrophotometer and absorption spectra were recorded using a Cary 15 spectrophotometer. Radioactivity was estimated in a low background Nuclear Chicago gas flow counter.

Freshly prepared proflavine hydrochloride solutions (1 mg/ml in 0.1 M potassium phosphate, pH 7.8) were freed of the undissolved residue by centrifugation prior to use. For the estimation of proflavine uptake, 2.5 ml samples of the culture containing the drug were withdrawn, chilled and centrifuged in the cold (10 min at 20,000 *g*). The sediments were suspended in 2.5 ml of cold 0.1 M potassium phosphate, pH 7.8, centrifuged, the colourless supernatants discarded, and the cells were resuspended in 2.5 ml of the same buffer solutions. These cell suspensions were disrupted by ultrasonic treatment and clarified by centrifugation (15 min at 27,000 *g*). Proflavine was estimated in the supernatans by measuring their absorbance at 444 nm.

RNA synthesis was followed by the incorporation of uracil-^{14}C into trichloroacetic acid (TCA) precipitate. 0.5 ml samples of the labelled uracil containing culture were added to 0.5 ml aliquots of ice-cold 10% TCA. The precipitates were collected on Whatman GF/C filters previously soaked in 5% TCA and washed twice on the filters with 5 ml of cold 5% TCA. The filters were placed on planchets, dried and analyzed for radioactivity.

Protein synthesis was estimated by the incorporation of L-leucine-^{14}C into hot TCA precipitate. 1.0 ml samples of the labelled leucine containing culture were added to 1 ml aliquots of 10% TCA and the mixtures were heated in a water bath for 15 min at 85–90°. After this step the procedure was essentially similar to the one used for uracil incorporation.

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3. Results

While investigating the role of cAMP on the synthesis of tryptophanase by *E. coli* we observed that the yellow colour of a proflavine solution was slightly altered on addition of cAMP. The difference absorption spectrum of proflavine (0.1 mg/ml) minus proflavine together with cAMP (5 mM) showed a peak around 410 nm and a trough around 470 nm, indicating a shift towards longer wavelengths of the absorption maximum of proflavine (444 nm) which probably reflects a chemical interaction between the drug and

Table 1
Effect of cAMP on the uptake of proflavine by *Escherichia coli* cells.

Incubation time (min)	Proflavine uptake (μg/ml culture)	
	Without cAMP	With cAMP
0.5	1.9	0.5
5.0	2.8	0.8

Proflavine (0.1 mg/ml) and cAMP (5 mM) were added at zero time and samples were withdrawn as indicated. Other conditions are described in sect. 2.

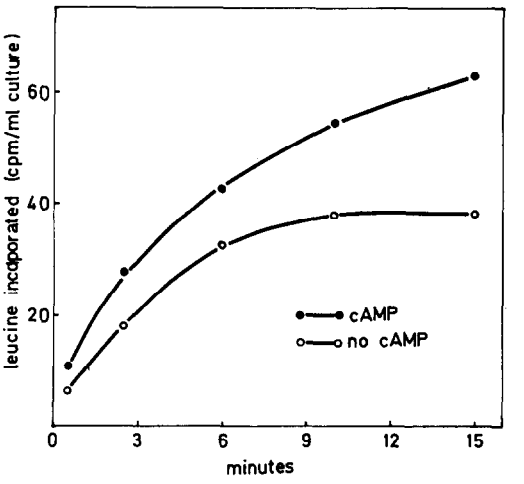

Fig. 2. Effect of cAMP on the residual incorporation of leucine after proflavine addition. L-leucine-^{14}C (0.1 mM, 0.2 mCi/mmole), proflavine (0.1 mg/ml) and cAMP (5 mM) were added at zero time. Other conditions are described in sect. 2.

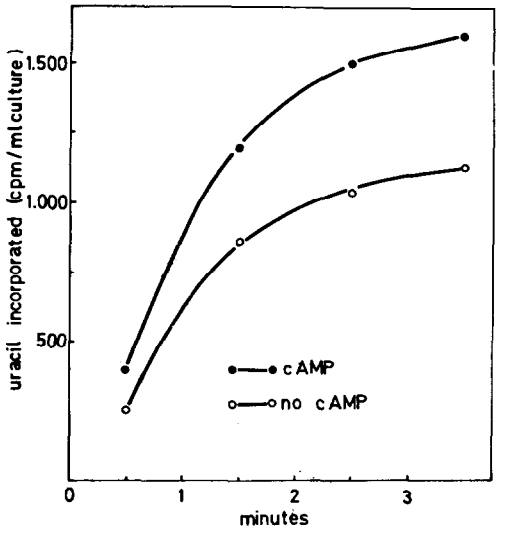

Fig. 1. Effect of cAMP on the residual incorporation of uracil after proflavine addition. Uracil-^{14}C (0.55 μM, 60 mCi/mmole), proflavine (0.1 mg/ml) and cAMP (5 mM) were added at zero time. Other conditions are described in sect. 2.

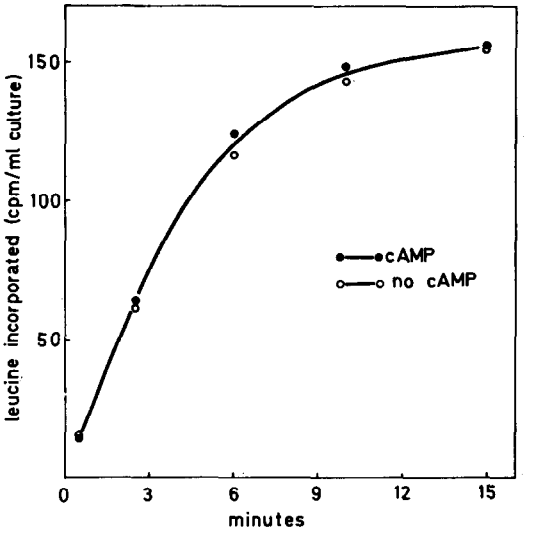

Fig. 3. Effect of cAMP on the residual incorporation of leucine after rifampicin addition. L-leucine-^{14}C (0.1 mM, 0.2 mCi/mmole), rifampicin (0.3 mg/ml) and cAMP (5 mM) were added at zero time. Other conditions are described in sect. 2.

the nucleotide. These observations suggested that cAMP might influence the uptake of proflavine by *E. coli* cells. As seen from table 1, 30 sec after the addition of proflavine and cAMP the amount of the drug which remained bound to the cells in the presence of cAMP was only about 30% of that bound in the absence of the nucleotide. Even after five min the uptake of the drug was inhibited by cAMP to the same extent.

To check whether the effect of cAMP on proflavine uptake altered the ability of the latter to inhibit RNA synthesis, we studied uracil incorporation in the presence of both the dye and the nucleotide. A rapid decrease in TCA precipitable counts has been observed with proflavine when uracil was present before the inhibitor [2]. However, some uracil was still incorporated when uracil and proflavine were added at the same time; fig. 1 shows that cAMP increased the rate of RNA synthesis under these conditions. Since the overall synthesis of RNA is not stimulated by cAMP in uninhibited cultures [7], the effect of cAMP on the inhibition by proflavine is very likely the result of a decrease in the incorporation of the inhibitor in the presence of the nucleotide (table 1).

In order to determine whether cAMP had any effect on protein synthesis in cultures inhibited by proflavine we studied the kinetics of leucine incorporation in the presence and absence of the nucleotide. As seen from fig. 2 leucine incorporation was about 150% greater in the presence than in the absence of cAMP. Although these results appear to be a consequence of a decrease in the inhibition of RNA synthesis by proflavine in the presence of cAMP (fig. 1) this could have been also due to the overall stimulation of protein synthesis by cAMP at the level of the polysome. Such a stimulation has not been detected in growing cells [7] but it could occur when RNA synthesis was impaired. To test this possibility we studied the kinetics of leucine incorporation after inhibiting RNA synthesis with rifampicin. This drug binds the bacterial RNA polymerase and seems to block one of the initial steps of transcription in cell-free extracts [8] as well as in intact cells [9]. The results, presented in fig. 3, show no difference in the residual incorporation of leucine in the presence or absence of cAMP and support the postulate that cAMP does not stimulate protein synthesis when the bulk of RNA synthesis is inhibited. It appears, therefore, that the enhancement of leucine incorporation by cAMP observed in the presence of proflavine (fig. 2) was due to the impaired drug uptake caused by the nucleotide.

4. Concluding remarks

cAMP plays an important regulatory role in the normal expression of catabolite repression sensitive bacterial genes [10]. The stimulation of enzyme synthesis by cAMP after the blockage of transcription by proflavine has been taken as a proof that cAMP is required at a translational step [4, 5]. However, our findings suggest that the effect of cAMP after proflavine addition is not related to the mechanism of catabolite repression. Therefore proflavine should not be used together with cAMP unless the particular experimental conditions ensure that the interference is eliminated.

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