Inhibition and stimulation of the development of the bioluminescent system in *Beneckea harveyi* by cyclic GMP

(cyclic nucleotides/bacterial bioluminescence/luciferase regulation)

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Communicated by Jack L. Strominger, September 27, 1976

**ABSTRACT**

The development of the luminescence system in *Beneckea harveyi* is controlled by cyclic nucleotides at the level of transcription. In the wild type, it is repressed by exogenously added guanosine 3':5'-cyclic monophosphate and this repression is overcome by the addition of adenosine 3':5'-cyclic monophosphate. These observations alone support a model in which these nucleotides act antagonistically. On the other hand, in a mutant requiring adenosine 3':5'-cyclic monophosphate for maximum luminescence, guanosine 3':5'-cyclic monophosphate stimulates the synthesis of the luminescence system at low concentrations and inhibits it at higher concentrations. These results are apparently not consistent with a model involving a simple antagonistic effect of guanosine 3':5'-cyclic monophosphate on the action of adenosine 3':5'-cyclic monophosphate.

The luminous bacteria offer a particularly attractive system in which to study regulatory effects of cyclic nucleotides. The system is inducible and subject to glucose repression, and this is reversed by exogenously added adenosine 3':5'-cyclic monophosphate (cAMP) (1, 2). The effect is at the level of luciferase synthesis, the activity of which can be monitored in *vivo* with high sensitivity and a great dynamic range of measurement. Mutants with one millionth of the wild type activity can be easily and quantitatively measured both in *vivo* and in *vitro* (3, 4).

We have utilized this luminescence system to study regulatory effects of cyclic nucleotides. In the wild type, the synthesis of the luminescence system is inhibited by exogenously added guanosine 3':5'-cyclic monophosphate (cGMP), indicative of an antagonistic relationship between cAMP and cGMP. However, in a mutant that requires cAMP for maximum luminescence (5), and evidently lacks or has very low levels of internal cAMP, low concentrations of cGMP stimulate while high concentrations inhibit the development of the luminescence system.

**MATERIALS AND METHODS**

*Beneckea harveyi*. Strain MAV, no. 392 (6, 7) was used as the wild type. A mutant (UY-437), requiring the addition of cAMP for bioluminescence, was previously described (5). This mutant is pleiotropic, and lacks capacity to luminesce, to form flagella, and to utilize certain carbohydrates for growth. All these deficiencies could be corrected by added cAMP.

Cells were grown with shaking at 27°C in a medium containing 0.5% yeast extract (Difco), 0.5% peptone (Difco), and 3% NaCl, at pH 7.2. The solid medium contained 1.5% agar (Difco). Growth was assayed by measuring the turbidity of the culture in a Klett-Summerson colorimeter with a no. 42 filter (100 Klett units equalled 2 X 10⁶ cells ml⁻¹). For bioluminescence measurements, aliquots of 0.5 ml were taken and assayed using a photomultiplier photometer. Light intensities are expressed in light units; one light unit equals 2.4 G quanta sec⁻¹ as established by standards of Hastings and Weber (8). *In vitro* luciferase activity was determined in cells treated with toluene, by using the assay described by Nealson et al. (1). cGMP and cAMP were purchased from Sigma.

**RESULTS AND DISCUSSION**

The luminescence system in bacteria is inducible, attributable to a substance ("autoinducers") released into the medium by the bacteria themselves (1, 9). During the time of induction, the luminescence increases 100-fold or more within the period of one generation (1, 3, 5). An inhibition by cGMP of this induced development of the luminescence in the wild type is shown in Fig. 1; there was no effect on growth. The inhibition, about 70%

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*Abbreviations: cAMP, adenosine 3':5'-cyclic monophosphate; cGMP, guanosine 3':5'-cyclic monophosphate.

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at a cGMP concentration of 0.3 mM, was reversed by cAMP, and suggested antagonism between the two nucleotides similar to that seen in other systems.

However, in a mutant which requires cAMP for the development of maximum bioluminescence (5), cGMP actually stimulates luminescence at very low concentrations (7 µM), while inhibiting at higher concentrations (Fig. 2A). These data are not consistent with a simple antagonistic model, and indicate that there is an effect of cGMP which is distinct from its antagonism with cAMP.

If exogenous cAMP (50 µM) is added to a growing culture of the mutant, then the development of luminescence is markedly stimulated (Fig. 2B). Under these conditions cGMP no longer stimulates at lower concentrations; only inhibition can be demonstrated. At even higher concentrations of cAMP (0.5 mM) (Fig. 2C), the mutant is even less sensitive to cGMP inhibition, and reacts very much like the wild type. The level of stimulation or inhibition in these experiments is shown in the inset to Fig. 2. We considered the possibility that the stimulation might be due to a contaminant. If so, it would have to be some compound other than cAMP, for in the experiments of Fig. 2B low levels of cGMP only inhibit in the presence of cAMP.

With the cAMP requiring mutant, either nucleotide can exert its competitive effect at any time during the growth cycle, irrespective of the time or order of addition. This shows that the effect of either nucleotide can be reversed, and supports the idea that nucleotide fluxes during growth, either singly or in tandem, can be important regulatory signals.

It was important to determine whether or not the inhibitory effect of cGMP on the luminescence is due to a block in the synthesis of the enzyme luciferase. The results (Fig. 3) show that luciferase synthesis, which in the mutant is dependent on cAMP (5), is almost completely inhibited by 0.7 mM cGMP, in parallel with the inhibition of the in vivo luminescence. Added cGMP (up to 2 mM) had no effect on the activity of luciferase in extracts.

In mammalian systems, it was suggested (10) that cGMP acts as an antagonist of cAMP. In bacterial systems, competitive relationships between cGMP and cAMP were also demonstrated in vitro with respect to their binding to the cAMP receptor protein and their effects on transcription of the lac and gal operons (11, 12). More recently, it was reported (13) that cGMP inhibits the synthesis of β-galactosidase and tryptophanase in growing Escherichia coli cultures at the transcriptional level, and that for both the presence of a functional cAMP receptor protein is required. In both Escherichia coli and Bacillus licheniformis cells, a reciprocal relationship between the internal concentrations of cGMP and cAMP has been reported (14). However, some other data, obtained under various physiological conditions (15), conflict with this, and thus question the generality of this relationship.

In the luminous bacteria, the stimulation by lower concentrations and inhibition by higher concentrations of cGMP is difficult to account for in a model involving a single site for cyclic nucleotides. However, suitable models can be constructed.
by postulating the involvement of two separate nucleotide sites, either on a single or on two different proteins. With a single protein, positive control could be effected either by cAMP acting at its own site or, in its absence, by cGMP at low concentrations acting allosterically via its separate site. Higher concentrations of cGMP would act antagonistically at the cAMP site.

The two protein model is essentially similar except that positive transcriptional control would be mediated directly by the cGMP protein, not via the cAMP binding element. Although this has evident drawbacks, it does receive support from the results of Sun et al. (16), who have isolated a specific cGMP binding protein from Caulobacter crescentus. The recent report of Kurn and Shapiro (17) that Caulobacter mutants resistant to repression by cGMP derivatives exhibited a pleiotropic phenotype affecting cAMP mediated phenomena would appear to be equally compatible with both models; because both postulate that inhibitory levels of cGMP act at the cAMP site. In Beneckea, the present results are also compatible with both models; the cGMP action could be mediated by a separate site on the cAMP binding protein, which has been reported to be present in relatively high concentrations in this organism (18), or by a different cGMP binding protein.

A final possibility is that there are two independently controlled genomes responsible for synthesis of the luminescence system. This possibility has already been suggested by the fact that “dark” mutants are really only dim (19). If the minor luciferase component were positively controlled by cGMP, with the major one being positively controlled by cAMP and antagonized by cGMP, then it would account for the observed results.

We are grateful to Dr. Kenneth Nealon for helpful discussions. This work was supported in part by grants from the National Science Foundation (BM-74-23651) and the National Institutes of Health (GM-19536) to J.W.H. and from the Tobias Landau Foundation to S.U.


Strain MAV used in that study has now been classified as Beneckea harveyi (7) and is not Photobacterium fischeri, as was reported.