Inhibition of Morphogenesis in a Prokaryote by Cyclic Adenosine 3':5'-Monophosphate

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(Received 9 August 1976)

SUMMARY

The morphological sphere-rod-sphere changes in *Arthrobacter crystallopoietes* were altered by introducing cyclic AMP into the bacteria. The presence of this cyclic AMP delayed both the formation of the bacillus form and the change back into the coccus form. A specific phosphodiesterase inhibitor, theophylline, also delayed the bacillus formation and the final coccus formation. Cyclic GMP had no demonstrable effect.

INTRODUCTION

Arthrobacter crystallopoietes undergoes a morphogenic cycle involving a sphere to rod to sphere change in morphology. In the presence of an inducing agent, such as succinate, the cocci elongate to bacilli which change back to cocci at the termination of active growth. If grown in a non-inducing medium, such as glucose mineral salts, growth continues in the coccus form with no morphological change. Luscombe & Gray (1971, 1974) grew this organism under carefully controlled conditions in a chemostat and concluded that the change from coccus to rod morphology was merely the result of increased growth rate and that no morphogenic cycle was involved. However, there is evidence in the literature which suggests that morphogenesis is involved in this genus (Clark, 1972), and so whether the bacillus form is the result of rapid growth, or the rapid growth is the result of the morphogenic changes associated with bacillary formation, remains to be resolved.

While the functions of cyclic AMP in prokaryotic cells are diverse and numerous (Rickenberg, 1974), morphogenic effects have been demonstrated in relatively few cases. These effects are mostly 'positive' and include such observations as the requirement of cyclic AMP in flagellation in coliforms (Yokota & Gots, 1970), the induction of fruiting body formation in *Myxococcus xanthus* (Campos & Zusman, 1974) and the enhancement of differentiation in *Caulobacter crescentus* (Shapiro *et al.*, 1972). This paper reports the inhibition of morphogenesis in *A. crystallopoietes* (Ensign & Rittenberg, 1963) by cyclic AMP, a 'negative' effect on a morphogenic event, demonstrating diversity in the regulatory roles of cyclic AMP in these prokaryotic systems. In addition, the effect of inhibition of cyclic AMP-specific phosphodiesterase was studied; the observed effects were thought to result from the prevention of cyclic AMP destruction *in vivo* and thus an increase in intracellular levels.

METHODS

Cocci of *Arthrobacter crystallopoietes* (ATCC15481) were taken from a synchronous 24 h culture and treated with Tris/EDTA (Leive, 1965) to render them more permeable to cyclic AMP (Pastan & Pearlman, 1968). Treated cells were inoculated into flasks containing 0.5 % (w/v) glucose minimal salts medium (GMS) (Ensign & Wolfe, 1964) in which cells are coccoidal only, and into three media in which cells undergo morphogenesis: 0.5 % (w/v) L-asparagine minimal salts (AMS); 0.5 % (w/v) succinate minimal salts (SMS); and TGY broth (Baltimore Biological Laboratories). Initial absorbance was standardized to 0.025 at 485 nm. At the time of inoculation and at 2 h intervals thereafter, cyclic AMP or cyclic GMP (Sigma) was added to maintain cyclic nucleotide concentrations of 0.1, 0.5, 1.0, 2.0 and 5.0 mM. Growth was measured by absorbance at 485 nm and morphology was determined microscopically using preparations stained with 0.1 % (w/v) methylene blue.

Controls utilized media to which no cyclic nucleotide had been added and media containing nucleotides previously treated with phosphodiesterase. Similar experiments were performed adding 10 mM-theophylline to the four media containing the Tris/EDTA treated cells at various time intervals.

Experiments were repeated using bacteria grown to different stages of the 'life-cycle', i.e. early rods, mid-exponential phase rods and fragmenting rods reverting to cocci. These were treated with Tris/EDTA and reinoculated into their original media which had been titrated from an alkaline pH to the original pH of the medium (7.2). To these cultures 5.0 mM-cyclic AMP, 5.0 mM-cyclic GMP or 10 mM-theophylline was added and observation of the life-cycle was continued.

RESULTS

The Tris/EDTA treatment had no effect on the morphogenic cycle but a slightly higher maximum growth was achieved compared with that of untreated control cells. Without this treatment, the cells were apparently impermeable to cyclic AMP and theophylline (Pastan & Pearlman, 1968). Cyclic GMP did not affect the growth or morphogenesis of treated or untreated cells under any of the conditions used. The morphogenic cycle in the three media is shown in Fig. 1. Cells remain coccoidal in the GMS medium only. No effect of cyclic AMP was seen in GMS medium in which cells were coccoidal only, or in any medium in which the cyclic AMP concentration was below 1.0 mM, or when phosphodiesterase was used to break down the cyclic AMP before it was added to the medium.

With concentrations of 1 mM-cyclic AMP and above, elongation of the cocci was delayed and rods did not appear until approximately 16 h (Fig. 2). Fragmentation was also delayed and occurred at 20 to 22 h as compared to 18 to 22 h in the controls. However, if cyclic AMP was not added until after 8 h growth, it had no observable effects on the 'morphogenic cycle'. There are several possible explanations for this loss of effect. The compound could be unstable in the increasingly alkaline media which result during growth, a critical threshold level might have been reached intracellularly, cyclic AMP might be effective for only a short time span, or after 8 h the cells might no longer be permeable to exogenous cyclic AMP.

To test this last possibility, bacteria were allowed to reach a particular stage in the morphogenic cycle then treated with Tris/EDTA, reinoculated, and cyclic AMP added as described in Methods. These experiments demonstrated that once rod formation had begun exogenous cyclic AMP did not delay the remaining morphogenic stages of fragmentation and reversion to cocci.

Fig. 1. *Arthrobacter crystallopoietes* was grown in various media, with growth assessed by the increase in absorbance at 485 nm: □, tryptone, glucose and yeast (TGY) broth; ●, 0.5% L-asparagine with minimal salts (AMS); ○, 0.5% succinate with minimal salts (SMS); ■, 0.5% glucose with minimal salts (GMS). Morphological changes are described and drawn as viewed by light microscopy on preparations stained with 0.1% methylene blue.

Fig. 2. Inhibitory effects of either 3':5'-cyclic AMP or theophylline added at the beginning of the 'life-cycle' (time = 0) to bacteria treated with Tris/EDTA (Leive, 1965). A, Morphogenic cycle of control cells grown in SMS, AMS, TGY with or without the Tris/EDTA treatment; B, inhibition by 1 mM to 5 mM 3':5'-cyclic AMP in the same media; C, inhibition due to addition of 10 mM-theophylline in the same media.

Theophylline inhibits the phosphodiesterase specific for cyclic AMP and, furthermore, inhibits, *in vitro*, the phosphodiesterase isolated from *Serratia marcescens* (Okabayashi & Ide, 1970). It was used in these experiments to maintain intracellular cyclic AMP levels during the morphogenic cycle. In the presence of 10 mM-theophylline, cells grown in GMS remained coccoidal and grew at the same rate as the control.

Theophylline delayed rod formation approximately 2 to 6 h. Once rods were formed they failed to fragment and revert to cocci until 36 h whereas controls without theophylline fragmented at 18 to 22 h (Fig. 2). When theophylline was added to Tris/EDTA treated bacteria which had already reached the rod stage, fragmentation was delayed for 2 to 6 h. The rods then fragmented and reverted to cocci.

DISCUSSION

Our observations indicate that exogenous cyclic AMP inhibits the normal initiation of the morphogenic event of elongation of cocci to rods in the life-cycle of *Arthrobacter crystallopoietes* and that theophylline has a similar effect, as well as causing a long delay in the process of fragmentation of rods to cocci. No effect was observed as a result of exogenous cyclic GMP in these experiments. This does not imply, however, that this nucleotide does not have a possible regulatory function, as yet undetermined, during the morphogenic cycle.

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