PERIODIC CYCLIC AMP UPTAKE BY SYNCHRONOUSLY GROWN CELLS OF *NOCARDIA RESTRICTA* AND *ARTHROBACTER GLOBIFORMIS*

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1. Introduction

The presence of cyclic AMP (adenosine-cyclic) 3′,5′-monophosphate and of an adenylate cyclase (EC 4.6.1.1.) have been shown in *Nocardia* [1–3] and *Arthrobacter* [1,4–6]. In synchronized cultures of *Nocardia restricta*, the intracellular cyclic AMP level exhibited rhythmic oscillations with a periodicity equal to the generation time of the culture [7].

Cyclic AMP supplied exogenously to non-synchronized cultures of *Nocardia salmonicolor* released catabolite repression of isocitrate lyase by fumarate [8]; this seems to indicate that cyclic AMP can penetrate into the cells of *Nocardia*. The present work was undertaken to study its uptake.

We show here that exogenous cyclic AMP penetrates effectively into the cells of *Nocardia restricta* and *Arthrobacter globiformis* but only during a short period of the cell cycle, the phase of DNA replication.

2. Materials and methods

2.1. *Methods of culture and synchronization*

Nocardia restricta ATCC 14887 was grown aerobically at 28°C in synthetic liquid media containing sodium succinate 15 g/l, as in [7]. The cultures were synchronized by diluting in a fresh medium cells already in stationary phase [7]. This method is often used to synchronize bacterial populations and is known not to cause metabolic disturbances [9].

2.2. *Control of synchronous DNA replication*

Two methods were used.

(i) The measurement of cell DNA content by Burton's method [10] as in [7].

(ii) Pulsed incorporations of tritiated thymidine into DNA as follows: successive 1 ml samples of the culture were taken at 20 min intervals and promptly incubated aerobically at 28°C for 10 min in the presence of 2 μCi [*methyl*-^{3}H]thymidine (1 Ci/mmol, CEN Saclay) added under 0.1 ml. The incubation was stopped by addition of 3 ml 10% trichloracetic acid. After 30 min at 4°C, the sample was filtered through a Millipore filter (0.65 μm) and then washed carefully twice with 5 ml 10% trichloracetic acid. Radioactivity on the filters was measured by liquid scintillation counting.

2.3. *Measurement of exogenous tritiated cyclic AMP uptake by the cells*

Cyclic [^{3}H]AMP accumulation was measured by the Millipore filtration method: 0.5 ml aliquots of cultures were incubated for 5 min, aerobically at 28°C, with 0.5 μCi cyclic [^{3}H]AMP (Radiochemical Centre, Amersham). Final cyclic AMP was 1 mM. To terminate the reaction, the mixture was rapidly diluted with 2 vol. cold fresh culture medium, immediately filtered with membrane filters Millipore HAWP 0.45 μm) and washed twice 2 ml fresh culture medium at 20°C. Dilution, filtration and washing procedures were conducted in less than 25 s. The filters were dried and the radioactivity was determined by scintillation counting. A sample taken immediately after addition of labelled cyclic AMP allowed the measurement of any radioactivity remaining trapped in intercellular fluid after washing. This 'blank' value was subtracted from all cellular radioactivity estimations.

3. Results

When *Nocardia restricta* cells taken from a synchronized culture were incubated in the presence of 1 mM cyclic [^{3}H]AMP, an increase in the total intracellular radioactivity was observed only during the phase of cell DNA replication (fig.1). This phase was determinated either by measurement of the variation cell DNA content or by pulsed incorporations of [^{3}H]thymidine into DNA. Heat-treated cells (100°C, 10 min) did not show any uptake of cyclic AMP. Aliquots of the extracellular medium from different incubated samples were chromatographed [11] and radioactivity present in migration areas of nucleotides and nucleosides counted. No detectable amounts of radioactive compounds other than cyclic AMP were found indicating that there was no breakdown of cyclic AMP in the extracellular medium after a 5 min incubation of cells in presence of nucleotide.

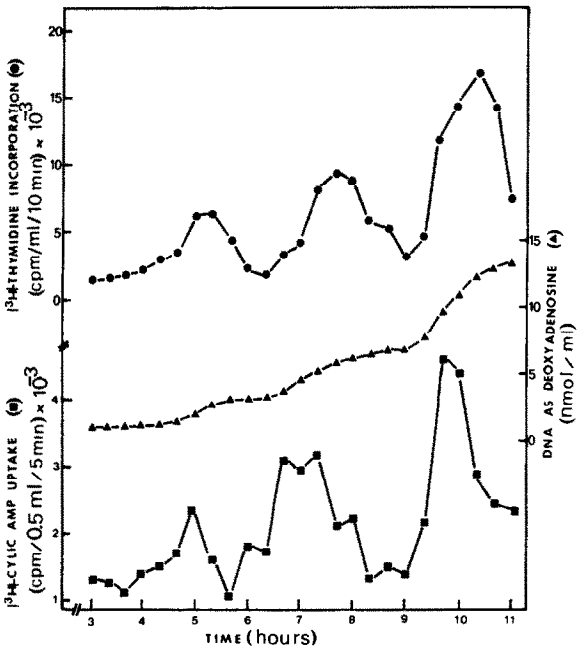

Fig.1. Periodic cyclic AMP uptake by synchronized *N. restricta* cells. Culture on succinate medium and synchronization were achieved as in [7]. Aliquots of cultures were drawn off every 20 min. The DNA replication was monitored on 1 ml aliquots by determination of [^{3}H]thymidine incorporation in acid insoluble fraction during 10 min (•) and by measurement of the cell DNA content by Burton's method (▲). Cyclic [^{3}H]AMP uptake by cells was measured on 0.5 ml aliquots over 5 min (■). Details are given in section 2. Each point is the average of 2 separate samplings assayed in duplicate.

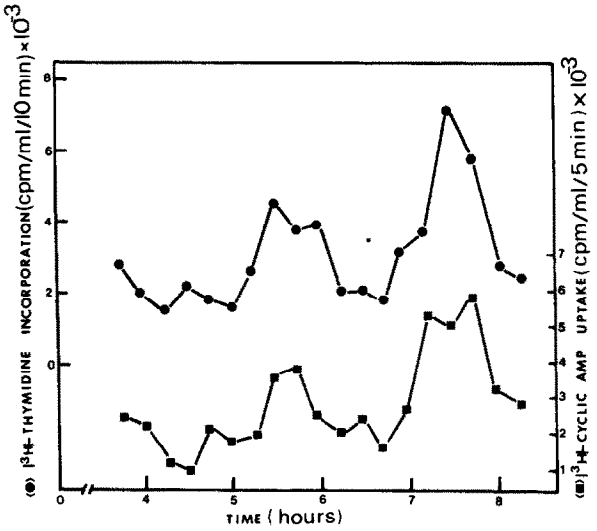

Fig.2. Periodic cyclic AMP uptake by synchronized *A. globiformis* cells. Culture and synchronization were achieved as in fig.1, but with 8 g/l glucose instead of succinate. Aliquots of cultures were drawn off every 15 min. The DNA replication was monitored by [^{3}H]thymidine incorporation (•) as in fig.1. Cyclic [^{3}H]AMP uptake was measured on 1 ml aliquots over 5 min (■). Each point is the average of 2 samplings assayed in duplicate.

To determine whether the radioactivity taken up by cells could be recovered from cell extracts as cyclic AMP, in one experiment, cells were allowed to take up cyclic AMP (5 mM; 2.5 μCi/ml) for 30 min before they were harvested by centrifugation and washed with fresh culture medium. The washed cells were broken by sonic disruption and the 30 min 20 000 X *g* supernatant of the cell extract was chromatographed [11]. The amount of radioactive cyclic AMP found in the spot corresponding to authentic cyclic AMP was determined by scintillation counting; 83% radioactivity taken up by the cells was found as cyclic AMP in the cell extract.

Synchronized cultures of *Arthrobacter globiformis* ATCC 8010 showed the same pattern of uptake of exogenous cyclic AMP: uptake was maximum during the replication of DNA and low after this phase (fig.2).

4. Discussion

Very little information is available concerning the process of cyclic AMP transport across plasma membranes although the importance of cyclic AMP excretion has been demonstrated for avian erythrocytes, a few mammalian cells maintained in culture, and bacteria [12–17].

If the uptake of cyclic AMP occurs only during a short period of the cell cycle of bacteria, as we have shown here in *N. restricta* and *A. globiformis*, it can predicted that in non-synchronized bacterial cultures, the maximum effect of the exogenous cyclic nucleotide will be observed after all cells have reached this specific period (i.e., after as long as one doubling time). Published data fit well with this hypothesis. *Arthrobacter crystallopoietes* takes up radioactive exogenous cyclic AMP 2 mM [4]; in this organism, addition of dibutyryl-cyclic AMP does not elicit any immediately noticeable effect; however, a correlation between premature reduction in growth rate and concentration of the cyclic nucleotide was observed [5]. To explain this complex effect, it was suggested that it involved changes in the cytoplasmic membrane structure and permeability. With the same organism it has been observed that the effect of cyclic AMP on cellular morphology is dependent on the time of addition of cyclic AMP to the culture [6]; it was concluded that cyclic AMP might be effective only for a short time span or that there might be changes in the permeability to exogenous cyclic AMP. We show here with synchronized cultures of *Arthrobacter globiformis* that cyclic AMP penetrates into the cells only during a short period of the cell cycle, the DNA replication phase.

Time-dependent effects of cyclic AMP on bacterial cells have been observed. The growth's inhibition of *E. coli* growing on gluconate by exogenous cyclic AMP, is not immediate, but begins only after the first doubling [18]. The inhibitory effect of cyclic AMP on uracil uptake in *E. coli* requires about one generation time to be evident [19]. The stimulation of active amino acids uptake by dibutyryl-cyclic AMP in *Streptomyces hydrogenans* is not immediate and reaches its maximum only after ~1h incubation [20]. In all these cases the delayed effect of the cyclic nucleotide suggests a time-dependent change in membrane permeability or structure.

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