Effect of exogenous nucleotides on growth and photopigment synthesis in *Rhodopseudomonas capsulata*

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Adenine, cytidine and guanosine nucleotides were supplied to cultures of *Rhodopseudomonas capsulata* under aerobic heterotrophic and phototrophic growth conditions. Aerobic growth is not affected by exogenous nucleotides (up to 10 mM) whereas phototrophic growth is strongly inhibited by adenine but not by guanosine or cytidine nucleotides. During phototrophic growth there is an inverse relationship between the concentration of exogenous adenine nucleotides and photopigment synthesis. There are no statistically significant differences between the inhibitory effect of AMP, ADP and ATP on the growth rate and bacteriochlorophyll synthesis since adenine nucleotides are incorporated into the cell as AMP by means of the phosphoribosyl transferase system.

Bacteriochlorophyll synthesis *Exogenous nucleotide* *Inhibition* *Phototrophic bacteria*

1. INTRODUCTION

Synthesis of light-harvesting bacteriochlorophyll (Bchl) in phototrophic bacteria during anaerobic photosynthetic growth is inversely related to the incident light energy [1,2]. In a number of Rhodospirillaceae, anaerobic dark growth promotes high Bchl synthesis [3] whilst during dark aerobic growth pigment synthesis stops [4].

High levels of ATP (either exogenous or intracellular) appear to be correlated with a decrease in Bchl synthesis in *Rhodopseudomonas* [5–7]. Also based on the changes in ppGpp levels during light shifts it has been proposed that this nucleotide may play a role in the control of Bchl synthesis [8]. We have described that cAMP does not control the synthesis of photopigments in *Rhodopseudomonas capsulata*, since exogenous cAMP has no effect on pigment levels and we were unable to detect cAMP neither in the extracellular medium nor in the cells growing under phototrophic conditions [23].

To clearly establish the relationship between nucleotides and photopigment synthesis, we have studied the effect of exogenous adenine, citidine and guanosine nucleotides under various growth conditions in *R. capsulata*. This study specifically shows that phototrophic growth and photopigment synthesis were strongly inhibited only by adenine nucleotides, and slightly by guanine nucleotides. However, we did not find statistically significant differences among ATP, ADP and AMP suggesting that they are cleaved during transport into the cell and that a common intermediary, probably AMP, is the effector in the control of pigment biosynthesis.

2. MATERIALS AND METHODS

2.1. *Bacterial strains*

A wild type of *Rhodopseudomonas capsulata* isolated from natural samples as in [23] was used.

2.2. *Media and growth conditions*

Aerobic growth was at 37°C in LB-rich medium [9]. Cultures were grown in 100 ml Ehrlenmeyer

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flasks containing 20 ml inoculated medium and shaken at 140 rev./min on a G-24 Gyratory shaker (New Brunswick Scientific Co.). For phototrophic growth, cultures were inoculated in completely filled screw-cap bottles containing Pfennig medium [10] supplemented with yeast extract (0.1%, w/v) and sodium acetate (0.2%, w/v). Cultures were illuminated with continuous fluorescent lamps (Excel, 15 W) at a light intensity of 3000 lux and incubated at 25°C. Growth was monitored by measuring the absorbance at 650 nm in a Coleman colorimeter.

2.3. *Photopigment analysis*

Cells were harvested at 8000 × *g* for 15 min and the pellet extracted with 5 ml 90% acetone over 24 h at 4°C. After centrifugation at 8000 × *g* for 15 min, the clear supernatant was assayed in a Pye Unicam SP-1700 spectrophotometer. The [Bchl *a*] was calculated from the absorbance at 773 nm using the specific absorption coefficient in acetone: ϵ_{sp} = 39.7 l/g.cm [11]. Carotenoids were quantified from the absorbance at 480 nm using an extinction coefficient: $E^{1\%}_{1cm}$ = 3000 [12].

2.4. *Chemicals*

Yeast extract and tryptone were purchased from Oxoid and nucleotides were obtained from Sigma.

3. RESULTS

3.1. *Effect of adenine nucleotides*

Fig.1 shows the effect of exogenous AMP on the aerobic and phototrophic growth in *R. capsulata*. Aerobic growth was not affected by exogenous AMP (⩽ 5 mM) whereas phototrophic growth was

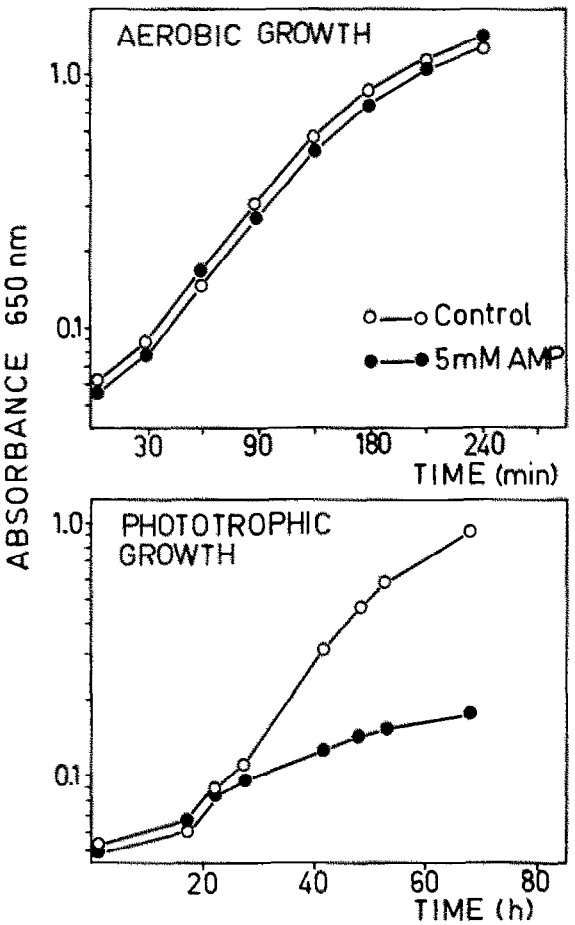

Fig.1. Effect of exogenous AMP in *Rhodopseudomonas capsulata*: (A) aerobic growth in LB rich medium, (B) phototrophic growth in Pfennig medium. Cultures without exogenous nucleotides were used as controls.

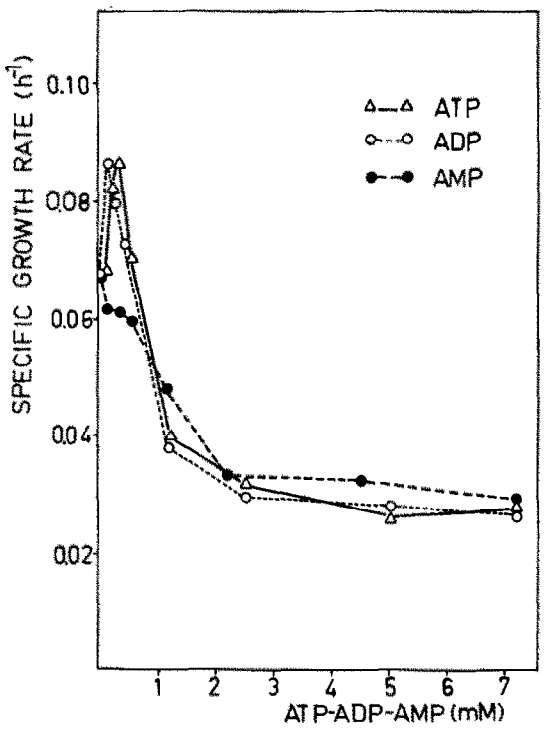

Fig.2. Relationship between specific growth rate and concentration of exogenous adenine nucleotides (AMP, ADP, ATP) in *Rhodopseudomonas capsulata* under phototrophic conditions.

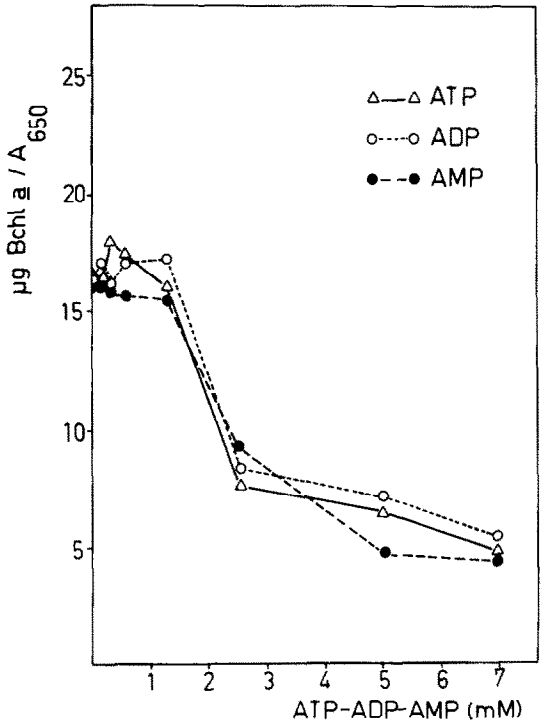

Fig.3. Relationship between specific pigment content (Bchl *a*) and the concentration of exogenous nucleotides in *Rhodopseudomonas capsulata* under phototrophic growth.

strongly inhibited by the same concentration, decreasing the growth rate > 2-fold. We have also studied the effect of addition of exogenous ADP and ATP, and also the same conclusions as with AMP were obtained when comparing aerobic and phototrophic growth.

Fig.2 shows the relationship between concentration of exogenous AMP, ADP and ATP and the growth rate during phototrophic growth. All 3 nucleotides inhibited photosynthetic growth and when results are compared by means of the ANOVA test we observed no statistically significant differences among the type of nucleotide, although they are significant between concentrations.

To establish if this inhibitory effect was due to a repression of Bchl synthesis, specific Bchl content was analyzed against exogenous nucleotide concentration (fig.3). Bchl levels were inversely correlated with adenine nucleotide concentrations but differences among the type of nucleotide were again not statistically significant. The same results were obtained for carotenoid pigments (not shown) indicating a common regulatory pattern in both sets of light-harvesting pigments.

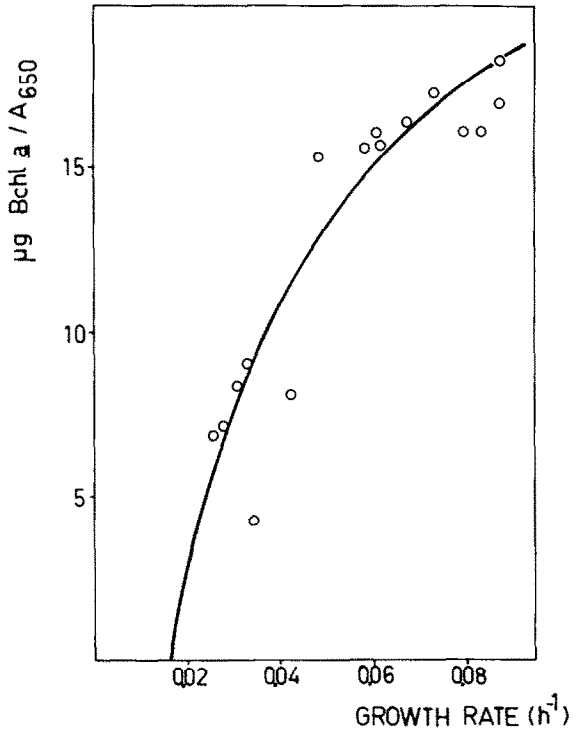

Fig.4. Relationship between growth rate and specific Bchl content in phototrophic growing *Rhodopseudomonas capsulata* under the effect of several concentrations of exogenous adenine nucleotides.

Table 1

Effect of addition of exogenous CMP, GMP and AMP on the growth rate and pigment biosynthesis in *Rhodopseudomonas capsulata* under phototrophic conditions

Treatment[a]	Doubling time (h)	Pigment content[b]	
		μg Bchl *a*/ A_{650}	μg Car/ A_{650}
Control	13.5	15.26	0.91
CMP	18.2	15.42	1.10
GMP	16.1	7.72	1.02
AMP	65.1	0.05	0.02

[a] Exogenous nucleotide = 2.5 mM

[b] *Abbreviations:* Bchl, bacteriochlorophyll; Car, carotenoids; A_{650}, absorbance at 650 nm

Consequently, taking into account that aerobic growth is not affected by exogenous adenine nucleotides it can be concluded that inhibition of phototrophic growth is due only to the decrease in pigment levels. When an inhibited culture (1.5 mM AMP) was shifted to high light intensity (9000 lux), growth rate increased again indicating that the lower growth rate observed was due to a decrease in light-trapping pigments (not shown).

3.2. *Effect of non-adenine nucleotides*

Non-adenine nucleotides, such as cytidine-5′-monophosphate (CMP) and guanosine-5′-monophosphate (GMP) were added to cultures of *R. capsulata* (table 1). At concentrations where there is a strong inhibition of growth and pigment synthesis by exogenous AMP (2.5 mM), we did not detect inhibition by exogenous CMP. GMP has no effect on the growth rate and carotenoid levels but reduced Bchl synthesis 2-fold when comparing with control.

4. DISCUSSION

One way to study the role of nucleotides in pigment synthesis is to perturb the intracellular balances by means of its exogenous addition to the cell. The incorporation of nucleotides into the cell is by means of a phosphoribosyl-transferase system. During the uptake, they are cleaved in the periplasmic space in Gram-negative bacteria, by means of 5′-nucleotidase resulting in nucleosides and free phosphate [13–15]. At the cytoplasmic membrane level, nucleosides are cleaved again to free bases by nucleoside phosphorylases and transported by membrane-associated phosphoribosyl-transferases that convert the bases into their nucleosides monophosphate [16,17].

Our results with exogenous nucleotides supplied to phototrophically growing *R. capsulata* are explained in this way since exogenous AMP, ADP and ATP have the same effect, both on growth and pigment synthesis. As can be deduced from fig.2,3, there are saturating kinetics between the effect and the concentration of adenine nucleotides suggesting a selective transport of these nucleotides across the membrane. Furthermore, independently of the degree of phosphorylation, nucleotides are cleaved during incorporation into the cell and returned again to the monophosphate form, thus producing the same effect on the growth and pigment synthesis in *R. capsulata*. Therefore, the interpretation that ATP alone inhibits Bchl synthesis in *R. sphaeroides* [7], *Rhodospirillum rubrum* [5] and *Chromatium vinosum* [18] can not be assumed from studies in which exogenous ATP is supplied to the cells. Unfortunately, in [5,7,18] complex fluctuations of cellular pools of ATP, ADP and AMP were obtained and did not provide convincing evidence in favour of a direct implication of ATP alone in the regulation of pigment synthesis. However, the accurate experiments in [19] with *C. vinosum* in response to light shifts do not support the role of ATP alone in controlling pigment synthesis.

Thus the role of ATP in controlling pigment biosynthesis in phototrophic bacteria remains unclear. One further possibility is based on the known effect of ppGpp as a common regulator of in vivo RNA formation and therefore may be an effector on Bchl synthesis [20]. In experiments with *R. sphaeroides* [8], an increase in ppGpp and a decrease in GTP was found after induction of photosynthetic apparatus by light shift-down. In accordance with that hypothesis is the fact that ppGpp levels in various non-phototrophic microorganisms increase during energy limitation showing an inverse relationship with growth rate [21].

Finally, the strict dependence of pigment and chromatophore synthesis on lipid, phospholipid and protein synthesis [22] also suggests a complex rather than simple regulation mechanism.

From these results, we conclude that only adenine nucleotides specifically inhibited phototrophic growth in *R. capsulata* as a consequence of the inhibition of Bchl and carotenoid synthesis. From the strong inhibition observed with exogenous AMP we cannot assume that ATP levels alone control chlorophyll synthesis in *R. capsulata*. Furthermore, the slight inhibition of Bchl *a* synthesis observed with exogenous GMP opens the possibility that a secondary control mechanism may exist at the transcriptional level, via ppGpp, but the main regulation mechanism occurs probably at the level of enzymatic activity in which adenine nucleotides play a central role.

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