Influence of Cyclic AMP on Photosynthetic Development in 
*Rhodospirillum rubrum*†

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During O₂-free growth in the light and in medium with pyruvate, *Rhodospirillum rubrum* exhibits diauxic growth. The cells first fermented pyruvate and afterwards photometabolized. Exogenous cyclic AMP acted to prolong the lag period between fermentative and photosynthetic development, as well as to slow the light-dependent growth rate. This observation, and in situ changes in the cyclic AMP levels in cells undergoing biphasic growth, suggested that the cyclic nucleotide was involved in photosynthetic differentiation, perhaps by repressing the formation of the bacteriochlorophyll needed to support growth in the light.

When grown anaerobically in the light, *Rhodospirillum rubrum* preferentially ferments sodium pyruvate and grows heterotrophically (13). Once pyruvate in the medium is used up, cell growth in cultures stops and then resumes photosynthetically when enough additional bacteriochlorophyll (Bchl) is formed. The resulting diauxic growth response is both pyruvate dependent and reversible. In addition to sodium pyruvate, cyclic AMP (cAMP) might also be involved in regulating diauxic photosynthetic development. This idea was based on the report (9) that the mode of growth in *R. rubrum* influences the levels of adenylate cyclase and 3',5'-cyclic nucleotide phosphodiesterase, the two enzymes involved in cAMP metabolism. Furthermore, increased levels of the cyclic nucleotide in cells of some photosynthetic organisms appear to affect Bchl (and chlorophyll) formation, as well as to inhibit certain photochemical reactions (4, 11, 17). As a result, experiments were undertaken to test the role of cAMP on *R. rubrum* development. In the present study, *R. rubrum* mutant C was used because the metabolic activities of these cells during the pyruvate-dependent diauxic growth response have been described previously (13). Diauxia also occurs in other strains of *R. rubrum*.

The typical diauxic growth response of *R. rubrum* mutant C is shown in Fig. 1. In the chemically defined pyruvate medium (6, 13), cells exhibited a pyruvate fermentative growth response with a generation time of 18.7 h. (We reported earlier [13] the extended generation time of mutant C in the chemically defined medium compared with growth in complex media. Regardless of the difference in growth rates, metabolic events in the cells are the same during diauxia under either growth condition.) After pyruvate fermentation, the lag period lasted 20 h before the resumption of growth photosynthetically (phase II growth). However, under virtually identical conditions, only with 10 mM cAMP added to the culture medium, the lag period before light-dependent phase II growth was lengthened more than twofold to 44 h. In the culture, cAMP seemed to principally influence the development and operation of photosynthetic activity after pyruvate was metabolized. Although the exogenously added cyclic nucleotide had no apparent influence on the pyruvate fermentative growth rate or basal levels of Bchl in cells grown without O₂, it seemed to act to slow secondary photosynthetic development by increasing the generation time up to 44 to 51 h from the 36 to 39 h measured in control cultures without cAMP (Fig. 1). Exogenously added cAMP has been recently reported (7) to similarly decrease the phototropic growth rate of *Rhodopseudomonas capsulata*.

The perturbation of pyruvate-dependent diauxia in *R. rubrum* seemed to be specific for cAMP. In separate experiments, the addition of 10 mM cyclic GMP extended neither the typical lag period between fermentative and photosynthetic metabolism nor the generation time of phase II photosynthetic growth (data not shown). Similarly, the addition of 10 mM AMP, ADP, or ATP to separate cultures had no effect on C cell diauxia. This fact suggested that the cyclic adenine nucleotide effect was a specific event and, in particular, that *R. rubrum* diauxia was not modulated by AMP, the degradation product of cAMP (10). In separate experiments, 10 mM N⁶,O⁴'-dibutyryl cAMP also failed to mimic the effect of cAMP on cell diauxia. The butylated compound has been reported (12) to be ineffective as well in regulation of cAMP-dependent β-galactosidase synthesis in *Escherichia coli*.

If cAMP is involved in the regulation of pyruvate-dependent *R. rubrum* diauxic cell development, anticipated changes in the level of the cyclic nucleotide should occur. To examine this, we measured both the extracellular and intracellular concentrations of cAMP in cultures during biphasic cell development under light conditions in pyruvate medium (Fig. 2). During pyruvate fermentative phase I growth, the extracellular concentration of cAMP averaged about 1.5 pmol/ml of culture fluid and only decreased slowly to 1.0 pmol/ml of culture fluid as the pyruvate concentration decreased (13) and phase I fermentative growth came to an end (Fig. 2A). Almost immediately thereafter, early in the lag period, the concentration of cAMP plunged to a very low basal level of about 0.2 pmol/ml of culture fluid, at which it stayed throughout the secondary photosynthetic growth period. Use of separate samples of phase I culture fluid and treatment with 3',5'-cyclic nucleotide phosphodiesterase (EC 3.1.4.17) (Fig. 2A) confirmed that the radioimmunoassay reaction was mainly measuring cAMP (2).

As anticipated from data on extracellular cAMP levels, the intracellular cAMP concentration changed in a similar way. The intracellular cAMP concentration ranged up to about 0.5 pmol/mg of protein during phase I fermentative cell development (Fig. 2B). However, in contrast to measurement of extracellular cAMP, the intracellular nucleotide level began

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ments of cAMP as a function of diauxic growth in C cells were measured with virtually identical results on three separate occasions in different experiments. However, the 35-h sample from the experiment shown in Fig 2B showed a transient increase in the intracellular cAMP content. Since this change was clearly detected only in the experiment shown, the value is not included with other data points. Nevertheless, it is retained to suggest to the reader that some fluctuation in in situ cAMP levels may occur in cells undergoing transition from pyruvate fermentation to photomethanogenesis. (In Fig 2B, cAMP determinations in cells during this transition are average values with a range ± 3 to 5% between duplicate samples.) A similar fluctuation in in situ cyclic GMP levels was reported during the developmental cycle in synchronous cultures of \(E.\) coli and Bacillus licheniformis (5). Clearly, the overall result of our measurements of intracellular cAMP in three separate experiments with \(R.\) rubrum mutant C argued that photosynthetic cell development and growth are accompanied by a decrease in cAMP. Conversely, elevated levels of the cyclic nucleotide favored pyruvate fermentation, presumably by repressing photosynthetic cell development. Such an idea seemed also to explain, in part, the effect of caffeine (a methylxanthine compound) on \(R.\) rubrum diauxie. In the presence of caffeine, a compound that might support an elevated in situ cAMP concentration by inhibition of 3',5'-cyclic nucleotide phosphodiesterase activity (8), it should be possible to influence cell diauxie. This was the case. In pyruvate medium prepared with 5 mM caffeine, the typical diauxic lag period was extended by more than 11%, from 17.5 to 19.5 h, and the ensuing phase II photosynthetic growth response was slowed down as the cell generation time increased to 31.5 h compared with 20.5 h in the control culture without caffeine addition (data not shown). Although the influence of caffeine on phase II growth (phase I fermentative growth was not affected) was more dramatic than the result of exogenous addition of cAMP to cultures (Fig 1), the prolongation of the lag period by enzyme inhibition was not. This could, however, be due in part to incomplete inhibition of 3',5'-cyclic nucleotide phosphodiesterase activity and a concurrent decrease in adenylate cyclase accompanying consumption of pyruvate in the medium. It was also noted that neither theophylline nor theobromine influenced \(R.\) rubrum diauxie. However, this result was not totally unexpected, since 3',5'-cyclic nucleotide phosphodiesterase activity in some other organisms is also not affected by either one of these methylxanthine compounds (1, 3).

FIG. 1. Effect of exogenous cAMP on diauxic growth response of \(R.\) rubrum mutant C (13). Anaerobic growth occurred in chemically defined pyruvate (0.1% [wt/vol]) medium in test tubes (15 by 160 mm), as described earlier (13), at 30°C in 2,130 lx (3.5 \times 10^4 ergs/cm\(^2\) per s) white light illumination. One optical density unit (15-mm path length) at 680 nm (OD\(_{680}\)) corresponded to about 3.5 \times 10^8 cells per ml. Growth with (C) and without (A) 10 mM cAMP.

FIG. 2. Changes in cAMP levels in \(R.\) rubrum mutant C cultures during diauxic development. Anaerobic growth in chemically defined pyruvate (0.15% [wt/vol]) medium at 30°C in Blake-type culture bottles (13). (A) Extracellular cAMP concentration in culture exposed to 4,307 \(1 \times (10^3\) ergs/cm\(^2\) per s\) white light. After removal of cells from 40 ml of culture solution with a Millipore membrane filter (average pore diameter, 0.22 \mu m), fluid samples were acidified with 0.1 M HCl, heated in a boiling water bath for 10 min, and lyophilized. Dried residue was suspended in 2 to 4 ml of 0.05 M sodium acetate buffer (pH 6.2), and cAMP was measured by the 

\[ ^{125} \text{I} \text{radioimmunoassay method} \] (14) (Becton-Dickinson Immunodiagnostics, Orangeburg, N.Y.). (B) Intracellular cAMP concentration in culture exposed to 2.027 lx (3.3 \times 10^4 ergs/cm\(^2\) per s) white light. Cells were collected from 50 ml of culture fluid by filtration as described above. The Millipore filter with cells was placed into 10 ml of 0.1 M HCl and treated for 10 min at 100°C. After acid extraction, cell debris was removed by centrifugation (5,000 \(\times g\), 10 min, 4°C), and the supernatant solution was lyophilized. The cAMP content in the dried residue was measured as described above. Symbols: (C), culture turbidity (one optical density unit at 680 nm [OD\(_{680}\)] corresponds to about 3.5 \times 10^8 cells per ml); (B), cAMP concentration; (A), control, cAMP content after treatment of sample with 80 \mu g of 3',5'-cyclic nucleotide phosphodiesterase for 17 h at higher temperature.
The effect of cAMP on pyruvate-dependent diauxie in *R. rubrum* is complex. In *R. rubrum*, as well as in other related phototrophic bacteria, exposure to exogenous cAMP caused a decrease in the rate of photosynthetic growth. However, in contrast to results with *Rhodopseudomonas capsulata* (7), the cyclic nucleotide seemed additionally to influence formation of Bchl in *R. rubrum*. This second, and perhaps more important, effect of cAMP occurred during the lag period in diauxie at which mutant C normally began to form additional Bchl before the resumption of cell growth photosynthetically. In cultures with added cAMP, both the onset of Bchl formation was delayed and the rate of pigment synthesis was slowed to about 20 pmol/mg of protein per h, or less than one half the rate in the control culture. (A sustained low rate of pigment synthesis could also explain the effect of cAMP on slowing cell growth.) On the basis of this observation, and the presence of low basal amounts of photochemically active Bchl in cells grown without O₂ (16), we suggest that cAMP, together with pyruvate fermentative metabolism, can directly regulate the formation of ICM₁, that fraction of photosynthetic intracellular membrane material in cells under apparent synthetic control by light intensity (13). Furthermore, it is believed that cAMP may as well influence related components in the photosynthetic apparatus of *Rhodopseudomonas gelatinosa*, which exhibits CO-dependent diauxie (15), and *Rhodopseudomonas capsulata* (unpublished data). Clearly, a role for cAMP must remain circumstantial, until the cyclic nucleotide can be shown to control synthesis or regulation (or both) of the photosynthetic apparatus on the molecular level. Present results, however, suggest that new insight into photosynthetic development and cellular differentiation will be forthcoming from these studies.

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