Plant Cell Physiol. 30(6): 911–914 (1989)

cAMP in *Anabaena cylindrica*: Rapid Changes in Cellular Levels in Response to Changes in Extracellular Environments

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The cellular level of cyclic 3′,5′-AMP (cAMP) in the cyanobacterium *Anabaena cylindrica* changed transiently in response to changes in extracellular environments. When the cells were transfered from dark to light, or anaerobic to aerobic conditions in the dark, the cAMP level rapidly decreased within one min and then gradually recovered. Addition of carbonyl cyanide *m*-chlorophenylhydrazone (CCCP) which inhibits ATP synthesis caused an increase in cAMP level in the light but not in the dark. The level of cAMP increased several fold by lowering the pH from 8 to 6. On the contrary, a rise of pH from 6 to 8 caused a decrease in the cAMP level. It is suggested that the change in membrane electrochemical potential is involved in the regulation of cellular cAMP concentration.

Key words: *Anabaena cylindrica* — cAMP — Cyanobacteria — Membrane potential.

As a second messenger, cAMP plays an important role in cell division and catabolite repression in bacteria (Pastan and Adhya 1976, Botsford 1981, Ullmann and Danchin 1983). Solaiman and Uffen (1984) have reported that photosynthetic cell development and growth of photosynthetic bacteria are accompanied by a decrease in cAMP. Conversely, elevated levels of the cyclic nucleotides favored pyruvate fermentation, presumably by repressing photosynthetic cell development.

In heterocystous cyanobacteria, the level of cAMP has been reported to change according to the nitrogen starvation prior to the heterocyst differentiation (Hood et al. 1979, Francko and Wetzel 1981). However, a short time cascade system for signal transduction as observed in animal cells has not yet been discovered in cyanobacteria. Recently, we have found that the cellular level of cAMP was transiently changed by a light-off or light-on signal in *Anabaena cylindrica*, i.e. a light-off signal caused a rapid rise in cAMP level and a light-on signal caused a rapid fall (Ohmori et al. 1988a). We have also found a protein showing GTP-binding activity in the crude extract from *Anabaena* cells containing plasma membrane and thylakoids (Ohmori et al. 1988b). It was suggested that the *Anabaena* cells has a cascade system responding to light signal.

It has been reported that the conditions which resulted in a temporary energy deficit brings about the major accumulation of cAMP in *E. coli*, and this elevated level serves as a signal for initiation of threonine dehydratase synthesis to supply energy by the nonoxidative degradation of threonine (Phillips et al. 1978). More recently, Mach et al. (1988) have reported that the cAMP level may be an intracellular indicator for an energy-starved state in *Bacillus subtilis* whatever the cause of energy limitation. This may be due to oxygen limitation, inhibition of ATP formation by dinitrophenol treatment, destruction of the proton motive force or glucose exhaustion by addition of α-methylglucose.

Cyanobacteria have acquired higher plant type photosynthesis and thus light is not merely a signal but also an energy source for ATP synthesis. A light driven H^+ translocation through the membrane is believed to couple with energy transduction in this organism. Energy supply by light may be a cause for the change in cellular cAMP level. In this experiment, the changes in cellular cAMP level in responce to the changes of external environment were determined. Particular attention was paid to the correlation between energy supply to the cell and cellular cAMP level.

Materials and Methods

Anabaena cylindrica (strain IAM M-1, Institute of Applied Microbiology, University of Tokyo) was grown to late logarithmic phase under nitrogen-fixing conditions in

Abbreviations: CCCP, carbonyl cyanide *m*-chlorophenylhydrazone; cAMP, cyclic 3′,5′-AMP; TCA, trichloroacetic acid; DCMU, 3,4-dichlorophenyl-1,1-dimethylurea.

the light (78 $\mu E \cdot m^{-2} \cdot s^{-1}$) at 28°C as previously reported (Ohmori and Hattori 1978). The cells were collected and washed once by centrifugation at 1,500 × *g* for 10 min. The sedimented cells were suspended in the fresh culture medium at concentrations of 10 to 15 μg Chl $\cdot ml^{-1}$. Then 30 ml of cell suspension was incubated for 40 min under white light (390 $\mu E \cdot m^{-2} \cdot s^{-1}$) or in darkness at 28°C with or without aeration. Light was supplied by an ELMO slide projector with a tungsten lamp. After the light was switched on or off, a portion of the cell suspension (2 ml) was removed at appropriate time intervals and mixed quickly with 0.22 ml ice-cold 50% TCA. To measure the amount of cAMP in the incubating medium, another 2 ml of the cell suspension was filtered correspondingly with a Millipore filter (HAWP, 0.22 μm in pore size) and the filtrate was mixed with 0.22 ml TCA. Those TCA-mixtures were kept at 4°C overnight. Then the TCA-insoluble material was removed by centrifugation and the TCA was removed by fractionating the mixtures with diethylether 4 times. The water phase containing cAMP was lyophilized and the concentration of cAMP in the dried samples was determined with a ^{125}I-radioimmnoassay technique using a Yamasa cAMP measuring kit according to Hasunuma and Shinohara (1985). The specificity of this method to cAMP was determined by breaking down the cAMP with cyclic nucleotide phosphodiesterase purchased from Sigma Co. (P-0134). The cellular cAMP concentration was calculated by subtracting the cAMP concentration in the incubating medium from that in the whole cell suspension. The concentration of cAMP in the incubating medium was less than 10% of that of cellular cAMP and it did not significantly change during the experimental period.

Results

When cells were exposed to light, the cellular cAMP level decreased rapidly within one min. Addition of DCMU, an inhibitor of photosynthetic electron transport, 5 min or 10 min before the illumination did not affect the light-induced decrease of the cAMP level (Fig. 1). It is suggested that the operation of the photosystem II-dependent electron transport system and thus light-dependent formation of a certain reducing substance such as thioredoxin was not involved in the light-induced change of the cAMP level. It was observed that in the dark the cAMP level fluctuated after the addition of DCMU. Besides the inhibitory effect of DCMU on light-driven electron transport, DCMU may also affect a certain dark reaction. The light-induced decrease in cAMP level was transient and the cAMP level reverted gradually in light (Ohmori et al. 1988a). The cAMP level under a light intensity of 390 $\mu E \cdot m^{-2} \cdot s^{-1}$ is usually kept below 100 pmol $\cdot$ (mg Chl)$^{-1}$.

When the cells were incubated in the dark and bubbled with Ar gas, cellular cAMP level was maintained at concentrations of 300 to 500 pmol $\cdot$ (mg Chl)$^{-1}$. The concentration of cAMP decreased rapidly to one tenth when the gas phase was changed from Ar to air (Fig. 2). Once decreased, the cAMP level gradually increased under aerobic conditions. Usually, the cAMP level recovered to a steady state 15 to 20 min after the Ar-to-air transition, however, the level was kept lower than that under the initial anaerobic conditions.

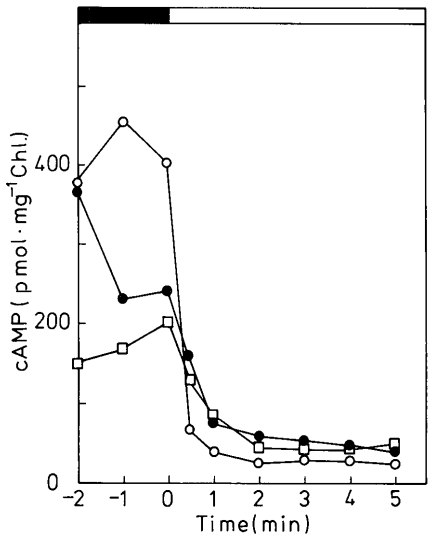

Fig. 1 Effect of DCMU on the cAMP level in *Anabaena cylindrica*. ○: control with no DCMU. ●: DCMU (2×10^{-5} M) was added 5 min before illumination, □: 10 min before illumination.

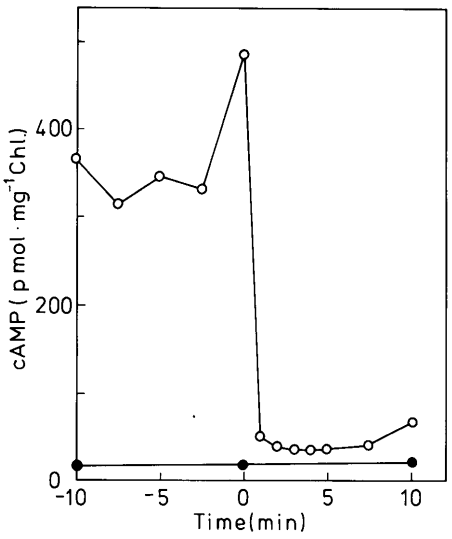

Fig. 2 Change in the cAMP level in *Anabaena cylindrica* caused by transferring the cells from anaerobic to aerobic condtitions. Air was introduced at time 0. ○: cAMP in the cell. ●: cAMP in the medium.

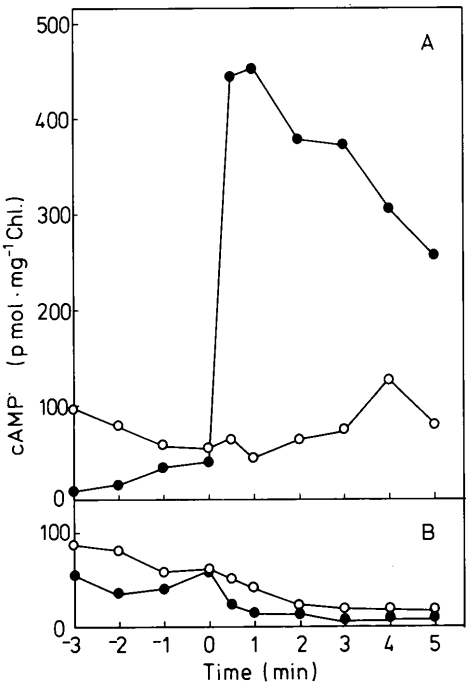

Fig. 3 Effect of CCCP on the level of cAMP in *Anabaena cylindrica* in the light (A) or dark (B). CCCP (2×10^{-5} M) was added at 0 min under different pH conditions. In (A), ●: pH 7.0, ○: pH 8.9. In (B), ●: pH 6.0, ○: pH 7.8.

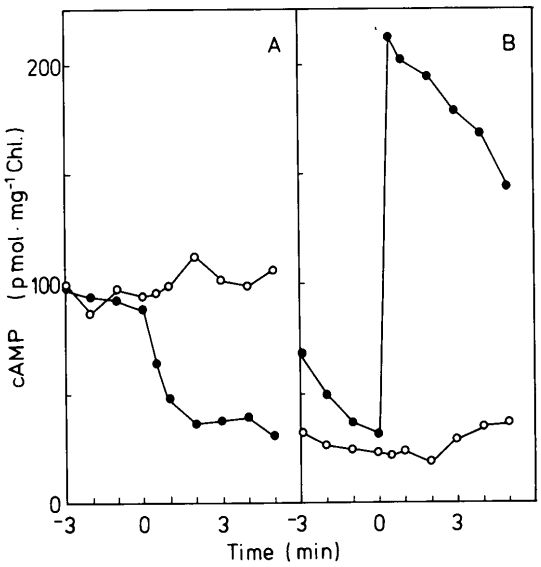

Fig. 4 Effect of pH changes in the medium on the cAMP level in *Anabaena cylindrica* in the dark. In (A), ○: Control at pH 6.0, ●: pH was shifted from 6.0 to 8.2 at 0 min. In (B), ○: Control at pH 8.3, ●: pH was shifted from 8.3 to 5.9 at 0 min.

Since the dark-to-light and anaerobic-to-aerobic transitions trigger photosynthesis and respiration, respectively, it is suggested that some environmental changes related to cellular energy production affect the cAMP level. To see the correlation between the activity of ATP synthesis and cAMP level, the effect of CCCP on the cAMP level was determined. When 2×10^{-5} M CCCP was added to the cell suspension in the light at pH 7, cellular cAMP level increased rapidly to about ten fold and reached 450 pmol·(mg Chl)$^{-1}$ after 30 s (Fig. 3). After that, the level gradually decreased but was kept fairly high [250 pmol·(mg Chl)$^{-1}$] even after 5 min. The effect of CCCP on the cAMP level was not observed at an alkaline pH of 8.9. Contrary to the case in the light, addition of CCCP in the dark at pH 7.8 or 6.0 caused no dramatic change in the cAMP level.

The inhibition of ATP synthesis by CCCP is attributed to its chemical nature as a protonophore which dissipates pH gradient through the membrane. The transmembrane pH gradient could also be changed by changing the pH of the extracellular medium. Thus the correlation between the change in transmembrane pH gradient and cAMP level was determined under dark conditions. When the cells were incubated first under low pH of 6.0 and then transferred to pH 8.0, the cellular cAMP level decreased (Fig. 4A). By changing the pH in the opposite direction, from pH 8.3 to 5.9, the cAMP level rapidly increased (Fig. 4B). The change in transmembrane pH gradient seems to be connected with the change in cellular cAMP concentration.

Discussion

The change in cAMP concentration in *Anabaena cylindrica* cells in response to the change in dark-to-light or anaerobic-to-aerobic conditions (Fig. 1, 2) can be understood on the same lines of Phillips et al. (1978) and Mach et al. (1988). A shift from dark to light or anaerobic to aerobic conditions in the dark caused a rapid rise in cellular ATP pool in *Anabaena cylindrica* and in other cyanobacteria (Bottomley and Stewart 1976, Ohmori and Hattori 1978, Nitschmann and Peschek 1986). Under these conditions, the cellular cAMP level decreased. On the other hand, an increase in cAMP level occurred when cellular ATP level is considered to be at a decrease. The assumption that the rapid cAMP production consumed much ATP resulting in the reduction of cellular ATP level cannot be accepted in this case because the ATP level (mM order) is about 1,000 times higher than cAMP level (μM order).

It is noticed that CCCP did not raise the cAMP level in the dark (Fig. 3B). Since CCCP inhibits ATP syntehsis both in the light and dark in *Anabaena* cells (Ohmori and Hattori 1978), the ineffectiveness of CCCP on the change in cAMP concentration suggests that the increase in cAMP cannot always be attributed to the decrease in cellular ATP level.

As earlier reported by Scholes et al. (1969) and later by Barsky et al. (1981), illumination of cyanobacterial cells causes a proton efflux in the cells. The proton efflux from cyanobacterial cells into the medium was also observed when cells were transfered from anaerobic to aerobic conditions in darkness (Scholes et al. 1969, Scherer et al. 1984). These proton translocation was assumed to be dependent on ATP produced by the photochemical reaction or by respiration. The electron transport did not directly couple with the proton efflux.

The rapid decrease in cAMP level as shown in Fig. 1 and 2 also occurred under conditions when the proton efflux would be enhanced by light or aeration. A rise in cAMP level by the addition of CCCP in the light under a low pH of 5.9 might be attributed to the dissipation of outward H^+ current. The intracellular pH of cyanobacteria has been reported to be 7.1 to 7.5 in the light and 6.7 to 6.9 in the dark (Falkner et al. 1976, Kallas and Dahlquist 1981, Ohmori et al. 1986). Under high pH conditions, addition of CCCP would not change the direction of H^+ flow from inside to outside of the cell. It is suggested that the change in H^+ gradient across the cytoplasmic membrane and the resulting change in the flow of H^+ through the membrane affects cellular cAMP level. The correlation between the change in plasma membrane potential and cAMP level has been reported in *Neurospora crassa* (Pall 1977). It can be considered that the change in the environment such as light, oxygen or pH causes the change in electrochemical force of membrane. This change in the membrane may somehow result in the change in the cellular cAMP level.

The author is grateful to Mr. L. Tupas for his critical reading of the manuscript, and to Miss. A. Ono and Miss. K. Abe for their skillful technical assistance. This work was supported by Grant-in-aid for Scientific Research (No. 63303011) from the Ministry of Education, Science and Culture of Japan.

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(Received January 27, 1989; Accepted June 19, 1989)