Impact of Inorganic Carbon Availability on Microcystin Production by Microcystis aeruginosa PCC 7806

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Batch culture experiments with the cyanobacterium Microcystis aeruginosa PCC 7806 were performed in order to test the hypothesis that microcystins (MCYSTs) are produced in response to a relative deficiency of intracellular inorganic carbon (Ci,i). In the first experiment, MCYST production was studied under increased Ci,i deficiency conditions, achieved by restricting sodium-dependent bicarbonate uptake through replacement of sodium bicarbonate in the medium with its potassium analog. The same experimental approach was used in a second experiment to compare the response of the wild-type strain M. aeruginosa PCC 7806 with its mcyB mutant, which lacks the ability to produce MCYSTs. In a third experiment, the impact of varying the Ci,i status on MCYST production was examined without suppressing the sodium-dependent bicarbonate transporter; instead, a detailed investigation of a dark-light cycle was performed. In all experiments, a relative Ci,i deficiency was indicated by an elevated variable fluorescence signal and led to enhanced phycocyanin cell quotas. Higher MCYST cell quotas (in the first and third experiments) and increased total (intracellular plus extracellular) MCYST production (in the first experiment) were detected with increased Ci,i deficiency. Furthermore, the MCYST-producing wild-type strain and its mcyB mutant showed basically the same response to restrained inorganic carbon uptake, with elevated variable fluorescence and phycocyanin cell quotas with increased Ci,i deficiency. The response of the wild type, however, was distinctly stronger and also included elevated chlorophyll a cell quotas. These differences indicate the limited ability of the mutant to adapt to low-Ci,i conditions. We concluded that MCYSTs may be involved in enhancing the efficiency of the adaptation of the photosynthetic apparatus to fluctuating inorganic carbon conditions in cyanobacterial cells.

Cyanobacteria are able to produce several metabolites that have hepatotoxic, neurotoxic, or dermatotoxic effects on humans and animals. Among this multitude of cyanotoxins, the hepatotoxic microcystins (MCYSTs) occur frequently. MCYSTs are cyclic heptapeptides synthesized by different genera of cyanobacteria, and Microcystis is the most prominent producer. So far, more than 70 different MCYST structures are known (5, 49). Many environmental studies have provided evidence that MCYSTs affect different trophic levels in lake ecosystems. Ecological and molecular studies have provided clues to regulation of MCYST production. Effects of nitrogen, phosphorus, trace elements, light, temperature, and pH on the MCYST content have been proposed and tested (23, 33; for an overview, see reference 46). According to other ecological studies, MCYSTs might act as iron-scavenging molecules or siderophores (for a review, see reference 15). Recent investigations have indicated that MCYSTs have a quorum-sensing or signaling function (8) or have a role as transmembrane transporters (35). Since a functional correlation between an MCYST and a mannan-binding lectin was revealed (22), MCYSTs might also contribute to the formation of colonies. However, the ecological function of MCYSTs remained nebulous.

A possible key for understanding the ecological function was the finding by Orr and Jones (35) that MCYST production is related to the rate of cell division. This finding implies that MCYST production should be controlled by internal cell processes related to growth and/or photosynthesis. From this conclusion, in turn, two consequences follow: (i) because growth and photosynthesis depend on a multitude of environmental factors, the presence of complex and multifactorial regulation of MCYST production is reasonable; and (ii) if MCYST production is influenced by growth or photosynthesis-related internal cell processes, one might speculate that MCYSTs are produced as regulatory agents in response to particular (adverse) growth or photosynthesis conditions.

Several findings indicate that MCYSTs have a putative role in regulation of photosynthetic processes. The influence of light (53, 54), the diurnal variation of the MCYST cell quota (2, 54), and the finding that MCYST production is largely controlled by pH (14) support such an assumption. Furthermore, differences in pigment content between a Microcystis wild-type strain and a mcyB mutant of this strain which is deficient in MCYST synthesis suggested that there is involvement in light adaptation processes (13). In addition, increased transcription of the mcyB and mcyD genes at high light levels was found (16). These findings are complemented by ultrastructural observations such as the high immunogold labeling densities of MCYSTs within the thylakoids (56) or carboxysomes (12). All these investigations (2, 12, 13, 14, 16, 35, 53, 54, 56) document that a putative function of MCYSTs related to photosynthesis is now more than just speculation. Regulation of photosynthesis is especially important in alkaline freshwater systems with high dynamics of inorganic carbon (Ci) concentrations (24) due to active Ci uptake by photosynthetic organisms.

An important prerequisite for successful growth in alkaline conditions...
environments is the ability of cyanobacteria to establish a carbon-concentrating mechanism (CCM) that enables them to concentrate C\textsubscript{i} more than 1,000-fold inside the cell (52). Cyanobacteria can adapt to fluctuating C\textsubscript{i} and O\textsubscript{2} conditions (42) by establishing the CCM in order to minimize carbon leakage through photospiration (6, 29, 32) or exudation (17) in the presence of low CO\textsubscript{2}/O\textsubscript{2} ratios. The CCM itself consists of two functional elements: carbon transport systems and containment of ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBiSCO) in carboxysomes. The uptake of C\textsubscript{i} occurs via independent transport systems, namely, two CO\textsubscript{2} uptake systems and two or more HCO\textsubscript{3}\textsuperscript{-} transporters. Species occurring in environments with fluctuating C\textsubscript{i} conditions exhibit the most complete set of transport systems (1). Most commonly, the dominating carbon species HCO\textsubscript{3}\textsuperscript{-} is transported by an HCO\textsubscript{3}\textsuperscript{-}/ATP binding cassette (ABC) transporter and an Na\textsuperscript{+}/HCO\textsubscript{3}\textsuperscript{-} symporter (19). Consequently, Na\textsuperscript{+} is required for the active transport of C\textsubscript{i} (20, 47). Moreover, carbon accumulation is regulated by light (21, 50) and hence also influenced by the diurnal light cycle.

The present study was undertaken to test the hypothesis that processes involved in cyanobacterial adaptation to fluctuating C\textsubscript{i} conditions and particularly to increased intracellular inorganic carbon (C\textsubscript{i,i}) deficiency are related to MCYST production. This is a novel hypothesis based on investigations cited above and our preliminary work. "Relative C\textsubscript{i,i} deficiency" refers to an internal CO\textsubscript{2}/O\textsubscript{2} ratio boosting the oxygenase function of RuBiSCO. Photosynthetic pigment concentrations, chlorophyll fluorescence, and pH were measured to characterize the responses of the cells to different degrees of C\textsubscript{i} accumulation. Different C\textsubscript{i,i} states were obtained by modification of the growth medium or by a diurnal dark-light change in the following experiments. In 1 and 2, the Na\textsuperscript{+} concentration was reduced to a minimum in order to constrain the C\textsubscript{i} uptake via the Na\textsuperscript{+}/HCO\textsubscript{3}\textsuperscript{-} symporter. Consequently, a relative C\textsubscript{i,i} deficiency was obtained with this treatment. In experiment 2, the responses to different C\textsubscript{i,i} conditions of the MCYST-producing wild-type strain and an MCYST-deficient mutant were compared. A third experiment tested whether the effects of the natural diurnal C\textsubscript{i,i} fluctuations on MCYST production resembled the findings obtained in experiments 1 and 2.

MATERIALS AND METHODS

Culture conditions. Cells of Microcystis aeruginosa strain PCC 7808 were grown in a 3-liter Erlenmeyer flask with 1.5 liters of culture medium at 18.5°C and a light intensity of 105 microeinsteins m\textsuperscript{-2} s\textsuperscript{-1} (experiments 1 and 3) or 30 microeinsteins m\textsuperscript{-2} s\textsuperscript{-1} (experiment 2). In order to study adaptation following the shift from dark to light, a cycle consisting of 15 h of light and 9 h of darkness was used in experiment 3. Cultures in experiments 1 and 2 were exposed to continuous light to exclude light-induced diurnal effects. Z/4 medium (57) was used in experiment 3. Cultures in experiments 1 and 2 were exposed to the shift from dark to light, a cycle consisting of 15 h of light and 9 h of darkness.

RESULTS

MCYST production in relation to different C\textsubscript{i} uptake conditions. Batch culture experiments were performed to investigate MCYST production in relation to relatively low (K medium) and high (Na medium) availability of C\textsubscript{i,i}. The variable fluorescence as a measure of C\textsubscript{i,i} deficiency within the cells was always higher in K medium (Fig. 1b). The differences in the variable fluorescence indicated that there was a significantly more pronounced C\textsubscript{i,i} deficiency in cells grown in K medium.

Sampling and analytical methods. At least 100 ml of a cell suspension was removed for analysis at each sampling time, and the volume was adjusted to obtain an approximately constant cell number. The pH values in cell suspensions were measured using a pH meter (pH 95; WTW Instruments). Major aliquots of the samples were filtered through several glass microfiber disks (GMF 5; Filtrak). The filters were stored at −18°C, freeze dried, and retained for chlorophyll a, carbon, and MCYST analyses. The filtrate was used to analyze the extracellular MCYSTs. A minor volume of the cell suspension (3 ml) was frozen without filtration to analyze phycocyanin. Cell numbers were determined after filtration onto polycarbonate filters (0.22 μm; Osmonics Inc.) by epifluorescence microscopy (Aniophot; Zeiss). The cell diameter was measured using an inverted microscope (Nikon Eclipse TE 2000-S) equipped with a digital camera (IVC TK-1380) and an image-processing system (analySIS 3.1 Build 540; Soft Imaging System GmbH). For Chl a analysis, cells were disrupted using an IKA Ultra TURRAX (T 18 basic; IKA Labortechnik) and extracted in ethanol (90%), and concentrations were determined based on emission at 667 nm using a luminescence spectrometer (LS 50 B; Perkin Elmer) and an excitation wavelength of 434 nm. For phycocyanin analysis cells were disrupted by freezing and ultrasonication (Sonopuls W 70; Bandelin) and extracted in phosphate buffer (pH 7.2, 4°C) (36), and concentrations were determined by measuring the emission at 645 nm using a luminescence spectrometer (LS 50 B; Perkin Elmer) and an excitation wavelength of 625 nm. The purity of phycocyanin was evaluated by using the A\textsubscript{436}/A\textsubscript{645} ratio (6, 29, 32) or exudation (17) in the presence of low CO\textsubscript{2}/O\textsubscript{2} ratios. The CCM itself consists of two functional elements: carbon transport systems and contain-

Calculation of growth and MCYST parameters and statistics. The growth parameters of the individual experiments were calculated using the following equation:

\[
X_t = \frac{X_{\text{max}}}{1 + (X_{\text{max}} - 1) \cdot e^{-\mu t}}
\]

where \(X_{\text{max}}\) is the maximum cell yield (capacity), \(\mu\) is the growth rate, \(t\) is time, \(X_t\) is the cell number at \(t > 0\), and \(X_0\) is the cell number at time zero.

All computations were performed using the R system for statistical computing (39).

MCYST production in relation to different C\textsubscript{i} uptake conditions. Batch culture experiments were performed to investigate MCYST production in relation to relatively low (K medium) and high (Na medium) availability of C\textsubscript{i,i}.
than in cells grown in Na medium (Table 1). Modification of
the $C_i,i$ availability resulted in changes in key growth param-
eters. Both the maximum growth rate ($\mu_{\text{max}}$) and $X_{\text{max}}$ were
reduced in K medium compared to Na medium (Fig. 1a). Cell
sizes, however, were only slightly different for the two treat-
ments (Table 1), thus allowing comparison of MCYST (and
pigment) cell quotas. The culture volumetric MCYST concen-
trations for the two treatments nearly reflected the corre-
sponding growth curve (Fig. 1c). More pronounced $C_i,i$
deficiency in cells grown in K medium resulted in enhanced cell
MCYST quotas (Fig. 1e and Table 1). The most obvious find-
ing of this experiment was the higher percentage of extracel-
lular MCYSTs in K medium (Fig. 1d). Thus, the total MCYST production (intracellular plus extracellular) was significantly
enhanced under $C_i,i$ deficiency conditions (K medium) (Fig. 1f
and Table 1). However, the ratio of MCYST-LR to [D-Asp]$^3$-
MCYST-LR was not influenced (Table 1). Besides the ele-
vated MCYST production, a higher phycocyanin content was
detected in cells grown in K medium (Table 1). No significant
differences were found between the two treatments with re-
spect to the Chl a content of the cells (Table 1).

**Responses of the wild-type strain and the mcyB mutant to
different $C_i$ uptake conditions.** To examine the potential im-
pact of MCYST on photosynthesis and/or growth, the effects of
$C_i,i$ availability on cell number (yield), photosynthetic pig-
ments, and variable fluorescence were compared for the
MCYST-producing wild-type strain *M. aeruginosa* PCC 7806
and its mcyB mutant lacking the ability to produce MCYSTs.
The effects of modification of the medium on the wild type and
mutant were tested by using analysis of variance (Table 2). A
significant interaction between the strain (as a proxy for the
ability or inability to produce MCYSTs) and the medium (as a
proxy for the Ci,i status of the cells) indicates the different responses of the wild type and the mutant to different Ci,i states. As anticipated by theory, the variable fluorescence reflected the Ci,i status of the cells, and no statistically significant strain-medium interaction was found (Table 2). Both wild-type and mutant cells exhibited higher variable fluorescence in response to enhanced Ci,i deficiency (K medium) (Fig. 2c). The two strains also did not respond significantly differently in their phycocyanin contents; both exhibited a significant increase in the high-Ci,i Na medium (Fig. 2a and Table 2). In contrast to the wild type, the mutant showed a clearly elevated concentration of Chl a with more pronounced Ci,i deficiency (K medium) (Fig. 2b).

**Intracellular MCYSTS during a dark-light change.** One may argue that limiting the Ci,i uptake in the K medium used in the two experiments described above might have represented rather artificial conditions for the *Microcystis* cells. Therefore, in a third experiment the impact of more natural variation of the Ci,i status on MCYST production was studied without suppressing the sodium-dependent bicarbonate transporter. For this purpose, samples of the culture were obtained at the end of the dark phase and in the following 15-h light phase (Fig. 3). The results for several variables indicate that there were three subphases of cellular response during the light phase (Fig. 3). The *Microcystis* cells grew exponentially over the entire experimental period (Fig. 3a). No distinct differences in the cell division rates between the three subphases of the experiment were observed (μ was 0.68, 0.63, and 0.56 day⁻¹ in subphases I, II, and III, respectively), which suggests that there was continuous cell division and excludes the possibility that there were large differences in cell size. Thus, an

### TABLE 1. Effects of relatively low deficiency (Na medium) and high deficiency (K medium) of Ci,i on different MCYST values, pigment contents, relative units of variable fluorescence, and cell size of *M. aeruginosa* PCC 7808 in batch experiment 1 (n = 3)

<table>
<thead>
<tr>
<th>Medium</th>
<th>MCYST cell quota (fg cell⁻¹)</th>
<th>MCYST production (fg cell⁻¹ day⁻¹)</th>
<th>MCYST-LR/ [D-Asp]³⁻</th>
<th>MCYST-LR ratio⁴</th>
<th>% of MCYST extracellular⁵</th>
<th>Chl a cell quota (fg cell⁻¹)</th>
<th>Phycocyanin cell quota (fg cell⁻¹)</th>
<th>Fv,rel (10⁻⁴ cell⁻¹)²</th>
<th>Cell diam (μm)⁷</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na (high Ci,i)</td>
<td>18.0 (0.95)</td>
<td>0.65 (0.3)</td>
<td>0.25 (0.01)</td>
<td>0.21 (0.01)</td>
<td>25.7 (3.5)</td>
<td>1.0 (0.02)</td>
<td>25.5 (0.14)</td>
<td>0.0007²</td>
<td>0.0001²</td>
</tr>
<tr>
<td>K (low Ci,i)</td>
<td>23.7 (0.96)</td>
<td>2.2 (0.3)</td>
<td>0.31 (0.03)</td>
<td>0.47 (0.03)</td>
<td>29.2 (1.6)</td>
<td>2.46 (0.16)</td>
<td>2.64 (0.15)</td>
<td>0.04²</td>
<td>0.065</td>
</tr>
</tbody>
</table>

*P* value 0.01² | 0.0001² | 0.08 | 0.0007² | 0.15 | 0.001² | 0.049² | 0.67² | 0.065²

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² See Materials and Methods for an explanation of the treatments. All values are averages for the exponential growth phase. The values in parentheses are standard errors.
³ A linear mixed model (36) with MCYST as the dependent variable and medium as the independent variable was used; the intercept was assumed to be constant, and only the slope for each replicate with time as a random effect was tested.
⁴ Distinct differences in the responses of the two strains were observed for the cell yield and Chl a content in subphases I, II, and III, respectively, which suggests that there was continuous cell division and excludes the possibility that there were large differences in cell size. Thus, an

### TABLE 2. Results of a two-factor analysis of variance in response to medium as a proxy for relatively high (K medium) and low (Na medium) deficiency of Ci,i and to strain (wild type and mutant) as a proxy for the ability or inability to produce MCYSTs

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Variable</th>
<th>df</th>
<th>Mean squares</th>
<th><em>P</em> value</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Microcystis</em> concn (cells ml⁻¹)</td>
<td>Strain</td>
<td>1</td>
<td>1.08 × 10¹⁰</td>
<td>0.05</td>
</tr>
<tr>
<td></td>
<td>Medium</td>
<td>1</td>
<td>1.13 × 10¹²</td>
<td>4.73</td>
</tr>
<tr>
<td></td>
<td>Strain × medium</td>
<td>1</td>
<td>2.24 × 10¹²</td>
<td>9.37</td>
</tr>
<tr>
<td></td>
<td>Residuals</td>
<td>8</td>
<td>2.39 × 10¹¹</td>
<td>.</td>
</tr>
<tr>
<td>Chl a concn (fg cell⁻¹)</td>
<td>Strain</td>
<td>1</td>
<td>7.47 × 10¹</td>
<td>4.89</td>
</tr>
<tr>
<td></td>
<td>Medium</td>
<td>1</td>
<td>1.32 × 10³</td>
<td>86.64</td>
</tr>
<tr>
<td></td>
<td>Strain × medium</td>
<td>1</td>
<td>5.15 × 10²</td>
<td>33.75</td>
</tr>
<tr>
<td></td>
<td>Residuals</td>
<td>8</td>
<td>1.53 × 10³</td>
<td>.</td>
</tr>
<tr>
<td>Phycocyanin concn (fg cell⁻¹)</td>
<td>Strain</td>
<td>1</td>
<td>2.11 × 10⁵</td>
<td>3.12</td>
</tr>
<tr>
<td></td>
<td>Medium</td>
<td>1</td>
<td>4.15 × 10⁵</td>
<td>6.14</td>
</tr>
<tr>
<td></td>
<td>Strain × medium</td>
<td>1</td>
<td>1.25 × 10⁵</td>
<td>1.85</td>
</tr>
<tr>
<td></td>
<td>Residuals</td>
<td>8</td>
<td>6.76 × 10⁴</td>
<td>.</td>
</tr>
<tr>
<td>Relative units of variable fluorescence related to equal cell concn</td>
<td>Strain</td>
<td>1</td>
<td>6.58 × 10⁻¹¹</td>
<td>1.97</td>
</tr>
<tr>
<td></td>
<td>Medium</td>
<td>1</td>
<td>1.85 × 10⁻⁹</td>
<td>55.38</td>
</tr>
<tr>
<td></td>
<td>Strain × medium</td>
<td>1</td>
<td>3.68 × 10⁻¹³</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td>Residuals</td>
<td>8</td>
<td>3.35 × 10⁻¹¹</td>
<td>.</td>
</tr>
</tbody>
</table>

⁵ All variables were measured in the exponential growth phase.
important prerequisite for comparing cell MCYST and pigment quotas was fulfilled. After the light period started, the pH increased distinctly in subphases I and II and remained almost constant during the rest of the experiment (subphase III) (Fig. 3b). The total intracellular carbon concentration decreased in subphase I and remained more or less low during subphases II and III (Fig. 3c). The cell MCYST quotas were variable over the diurnal time scale (Fig. 3f). They remained almost constant during the first 2 h of the light period (subphase I), and this was followed by a progressive increase in subphase II and a tendency toward a slight decrease in subphase III. Extracellular MCYSTs contributed less than 10% and thus contributed only marginally to the total MCYST concentration (data not shown). The variable fluorescence (as a measure of Ci,i deficiency within the cells) rose after the light period started, and it coincided during the rest of the experiment with variations in cell MCYST quotas (compare Fig. 3e and 3f). Coinciding with decreasing variable fluorescence in subphase III, the phycocyanin cell quotas also declined (compare Fig. 3 d and 3e).

**DISCUSSION**

The results of all three experiments reported here support our hypothesis that varying Ci,i conditions have an influence on MCYST production. In freshwater cyanobacteria, intracellular accumulation of Ci by the CCM is induced primarily by depletion of the Ci,i pool (55). Photorespiratory metabolites have also been discussed as further elicitors (18, 55). We adjusted the cellular status for relative low or high Ci,i deficiency by using two different media (Na medium and K medium) in experiments 1 and 2 and by the dark-light change in experiment 3. Increased Ci,i deficiency must be assumed for the K medium treatments in the first and second experiments and during subphase II in the third experiment. In all cases, increased Ci,i deficiency was indicated by elevated variable fluorescence and, to a lesser extent, by elevated cell phycocyanin quotas. These findings corroborate results of Mandori and Melis (26), Müller et al. (32), MacKenzie et al. (25), and Burns et al. (3) for phycocyanin (and the phycocyanin/Chl a ratio) and results of Miller and Canvin (28) and Crotty et al. (7) for the variable fluorescence. Remarkably, the mcyB mutant did not differ significantly from the wild type in this respect, indicating that the two strains were affected in the same way by modulating the extent of Ci,i deficiency irrespective of their ability to produce MCYSTs.

In all test designs, experimentally increased Ci,i deficiency led to enhanced MCYST production (higher cell MCYST quotas in the first and third experiments or elevated release of MCYST in the first experiment). A putative function of MCYSTs can be assumed inside or outside the cells. Our results for the relationship between relative Ci,i deficiency and MCYSTs cannot answer this question, as both intra- and extracellular levels of MCYSTs were enhanced under Ci,i-deficient conditions. Based on previous investigations, the occurrence of extracellular MCYSTs is ascribed to culture density or
may originate from senescence and cell death at the end of exponential growth (46). In contrast to these hypotheses, the percentage of extracellular MCYSTs in experiment 1 was considerably elevated during exponential growth. The interpretation that the latter finding is a result of directed transport of MCYSTs out of the cells is supported by the fact that an ABC transporter gene is directly linked to the \textit{mcy} gene cluster (8, 37, 51). Cyanobacterial ABC transporters may be involved in the transport of allocrites, including bicarbonate (34). Furthermore, since nodularin, another cyanotoxin closely related to MCYSTs, can form pores in lipid bilayers (48), MCYSTs were assumed to act as transmembrane transporters (35). A relationship between \textit{C\textsubscript{i,i}} deficiency and extracellular MCYSTs is further supported by the fact that, in general, the amount of extracellular MCYSTs seems to be low in continuous cultures (23, 54) and higher in batch cultures (14, 35, 41, 44). Because exposure to light and the culture regimen have an influence on the extent of \textit{C\textsubscript{i,i}} accumulation, all these findings may be interpreted as an effect of relative \textit{C\textsubscript{i,i}} deficiency.

The assumption that MCYSTs have an extracellular function competes with the finding that the bulk of MCYSTs is localized inside the cells (12). In detail, the bulk of MCYSTs was detected in intracellular inclusions, such as polyphosphate bodies and carboxysomes, without any dependence on the cells’ experience with light. Judging from the large amounts of MCYSTs embedded in carboxysomes, the hypothesis that MCYSTs might adjust the synthesis of RuBisCO was supported (12, 14). Other investigations have shown that the high-

![FIG. 3. Dark-light changes in a growing \textit{M. aeruginosa} PCC 7806 batch culture, including (a) cell concentration (note the logarithmic scale), (b) pH value and pH change (\(\Delta\) pH) between two consecutive sampling points, (c) total carbon concentration, (d) phycocyanin cell quota, (e) relative units of the variable fluorescence (\(F_{\text{v,rel}}\)), and (f) MCYST cell quota. All values are means ± standard errors obtained from three replicates. \(F_{\text{v}}\), variable fluorescence; \textit{def.}, deficiency; \textit{invar./decr.}, invariate/decreasing.](image-url)
and open squares, respectively) (54), and by using a batch culture of *M. panniformis* the increasing Ci,i deficiency during the transition from sub-proceeding carboxylation. We assumed that the cells sensed ciency in subphase III, in subphase I the Ci,i deficiency was low compare two phases with low Ci,i deficiency, subphases I and III. However, only in subphase III was the low Ci,i deficiency caused by elevated Ci,i accumulation. Unlike the low Ci,i deficiency in subphase III, in subphase I the Ci,i deficiency was low due to the transition from the dark phase to the light phase. However, cells became increasingly Ci,i deficient due to the proceeding carboxylation. We assumed that the cells sensed the increasing Ci,i deficiency during the transition from subphase I to subphase II and that this triggered MCYST production. As a result, Ci,i deficiency was reduced in subphase III. This postulated mechanism would also explain the diurnal pattern of MCYST cell quotas reported previously for *M. aeruginosa* in continuous cultures (54) and for *Microcystis panniformis* in batch cultures (2). Generally, all these experiments were characterized by an increase in the MCYST cell quota two or more hours after the light was switched on (Fig. 4). However, the amplitudes of variation differed in the individual investigations. This was likely due to differences in the culture regimen and cell densities, which may have generated different states of Ci,i deficiency.

Chl a quotas reacted differently to increased relative Ci,i deficiency in the MCYST-producing wild-type strain and the mcyB mutant. Whereas in the wild-type strain the Chl a cell quotas increased, the mutant showed no distinct enhancement. In experiments performed by Hesse et al. (13), the same wild-type strain also exhibited a higher Chl a content than the same mutant under light limitation conditions, which was comparable to the relative Ci,i deficiency in K medium in our second experiment (Fig. 2). In contrast to the response of Chl a cell quotas, both the wild type and the mutant showed increased phycocyanin cell quotas with increased Ci,i deficiency. Hence, the ratio of phycocyanin to Chl a remained almost unchanged in only the wild-type cells. Cyanobacteria are known to adapt to varying Ci conditions by adjusting the phycocyanin/Chl a ratio (10, 26). This adaptation to the prevailing Ci,i conditions facilitates energization of Ci uptake (21, 27) and Ci,i accumulation (31), thereby improving the photosynthetic efficiency (11) in the wild-type cells. In contrast to the wild type, this feature seems to be repressed or deactivated in mutant cells. Obviously, for this reason the mcyB mutant in experiment 2 showed lower cell yields with increased Ci,i deficiency compared to Ci,i-sufficient conditions. However, the effect of improving the photosynthetic efficiency and hence the cell yield by adapting the phycocyanin/Chl a ratio to the prevailing Ci,i conditions is light dependent. Under high-light conditions (experiment 1) the capacity of this mechanism was obviously exceeded, so that the cell yield with increased Ci,i deficiency even in the wild-type strain could not be fully compensated for to the same extent as under Ci,i-sufficient conditions (Fig. 1a). On the other hand, under rather low-light conditions (experiment 2) the adaptation of the wild-type cells resulted in very slight differences in cell yields between the two Ci,i variants. Since there was close agreement in all experiments between the ability to adjust the photosynthetic pigments to the Ci,i status and the extent of MCYST production, we concluded that MCYSTs might be involved directly or indirectly in Ci,i accumulation. Furthermore, based on the time pattern seen in experiment 3 it is proposed that MCYST production precedes Ci,i accumulation. As the ratio of the two MCYST variants (MCYST-LR and [d-Asp³]-MCYST-LR) produced by strain PCC 7806 was not influenced by the Ci,i status, the possibility of a specific function of one of these structural variants can be excluded.
All putative functions of MCYSTs should result in a competitive advantage for strains able to produce MCYSTs over non-producing strains unless the latter have developed compensating mechanisms. These compensating mechanisms may consist of producing other peptides as substitutes for MCYSTs (35, 43, 45) or adapting other physiological properties, leading to the same photosynthetic efficiency (e.g., by modulation of RuBisCO specificity [52]). Nevertheless, our hypothesis that MCYSTs might be involved in the adaptation to varying Ci,i conditions is one of the few existing explanations of the eco-physiological function of MCYSTs which are in accordance with recent insights into the early evolution of MCYST synthesis. As Rantala et al. (40) emphasize, “any model that attempts to explain why cyanobacteria make these peptides must now account for the age of the toxin synthetase genes.” Our experimental findings indicating that MCYSTs have a role in the photosynthetic adaptation to a relative Ci,i deficiency meet this requirement. A relative physiological Ci,i deficiency may have appeared during cyanobacterial evolution parallel to increasing environmental oxygen concentrations (4), causing an increasing oxygenase function of RuBisCO. An enhanced oxygenase function of RuBisCO, in turn, might have provided a competitive advantage for strains able to produce MCYSTs over others. Toxins in cyanobacteria and the ecological and molecular aspects of the inorganic carbon-concentrating mechanism in cyanobacteria. Plant Physiol. 17:124–126.

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sions beyond the scope of this study are necessary to test this hypothesis on the molecular level.

We thank Gabriele Egerer, Yvonne Calderelli, and Katharina Memmel for technical assistance and Emily Susko for linguistic improve-
ments. We are particularly indebted to Thomas Börner for providing

This study was supported by the German Science Foundation (DFG
grant Be 1671/1-4, 3).

Acknowledgments

We thank Gabriele Egerer, Yvonne Calderelli, and Katharina Memmel for technical assistance and Emily Susko for linguistic improvements. We are particularly indebted to Thomas Börner for providing the mcyB mutant and to Ingrid Chorus for valuable comments on a draft of the manuscript.

This study was supported by the German Science Foundation (DFG grant Be 1671/1-4, 3).

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