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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 ($C_{i,i}$). In the first experiment, MCYST production was studied under increased $C_{i,i}$ 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 $C_{i,i}$ 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 $C_{i,i}$ 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 $C_{i,i}$ 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 $C_{i,i}$ 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-$C_{i,i}$ 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 regulative 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 (C_i) concentrations (24) due to active C_i uptake by photosynthetic organisms.

An important prerequisite for successful growth in alkaline

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environments is the ability of cyanobacteria to establish a carbon-concentrating mechanism (CCM) that enables them to concentrate C_i more than 1,000-fold inside the cell (52). Cyanobacteria can adapt to fluctuating C_i and O_2 conditions (42) by establishing the CCM in order to minimize carbon leakage through photorespiration (6, 29, 32) or exudation (17) in the presence of low CO_2/O_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_i occurs via independent transport systems, namely, two CO_2 uptake systems and two or more HCO_3^- transporters. Species occurring in environments with fluctuating C_i conditions exhibit the most complete set of transport systems (1). Most commonly, the dominating carbon species HCO_3^- is transported by an HCO_3^- ATP binding cassette (ABC) transporter and an Na^+/HCO_3^- symporter (19). Consequently, Na^+ is required for the active transport of C_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_i conditions and particularly to increased intracellular inorganic carbon ($C_{i,i}$) deficiency are related to MCYST production. This is a novel hypothesis based on investigations cited above and our preliminary work. "Relative $C_{i,i}$ deficiency" refers to an internal CO_2/O_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_i accumulation. Different $C_{i,i}$ states were obtained by modification of the growth medium or by a diurnal dark-light change in the following ways. In experiments 1 and 2, the Na^+ concentration was reduced to a minimum in order to constrain the C_i uptake via the Na^+/HCO_3^- symporter. Consequently, a relative $C_{i,i}$ deficiency was obtained with this treatment. In experiment 2, the responses to different $C_{i,i}$ 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 $C_{i,i}$ fluctuations on MCYST production resembled the findings obtained in experiments 1 and 2.

MATERIALS AND METHODS

Culture conditions. Cells of *Microcystis aeruginosa* strain PCC 7806 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^{-2} s^{-1} (experiments 1 and 3) or 30 microeinsteins m^{-2} s^{-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 as the culture medium in experiment 3 and for the reference treatments in experiments 1 and 2. The concentration of $NaHCO_3$ in these reference treatments was 2 mM (Na medium). To restrict sodium-dependent bicarbonate uptake, this medium was modified for the $C_{i,i}$ deficiency treatments in experiments 1 and 2 by replacing all the sodium components with the corresponding potassium compounds (K medium). Cells were not washed and experiments were carried out in glass flasks, so a minimum amount of Na^+ always remained. Experiment 2 was performed to compare the adaptations to different $C_{i,i}$ conditions of the wild-type strain *M. aeruginosa* PCC 7806 and its knockout mutant lacking the ability to produce MCYSTs (9). The mutant was kindly provided by the laboratory of Thomas Börner (Institute of Biology, Genetics, Humboldt University, Berlin, Germany). All experiments were carried out with batch cultures, and the initial cell concentration for all treatments was adjusted to 10^5 cells ml^{-1}. Each treatment was performed in triplicate.

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* (Chl *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 (Axiophot; Zeiss). The cell diameter was measured using an inverted microscope (Nikon Eclipse TE 2000-S) equipped with a digital camera (JVC 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 (Sonoplus W 70; Bandelin) and extracted in phosphate buffer (pH 7, 12 h, 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_{620}/A_{280} ratio (30). Absorbance measurements were obtained using a UV/visible light spectrometer (Lambda 12; Perkin Elmer). For carbon analysis, a cell suspension was filtered onto precombusted glass microfiber disks (GMF 5; Filtrak), freeze dried, and analyzed with a carbon analyzer (Leco C 200; Leco Instrumente GmbH). Chlorophyll fluorescence as a measure of relative $C_{i,i}$ deficiency, which is equivalent to an inverse measure of $C_{i,i}$ accumulation, was determined with a PAM fluorometer (Walz) using the following procedure. Prior to measurement, cells were dark adapted for 20 min. After a weak pulse-modulated beam was switched on, the initial fluorescence was measured. The maximum fluorescence was determined following a saturating flash after exposure to actinic light. The variable fluorescence was expressed as the difference between the maximum fluorescence and the initial fluorescence (7, 28). To compare variable fluorescence values for various experiments and treatments, the measured values were related to the corresponding cell concentrations. Intracellular MCYSTs were extracted using methanol-1.3 mM acetic acid (67:33, vol/vol). The extracts were preconcentrated by solid-phase extraction (Strata C_{18}; Phenomenex Inc.). In order to analyze extracellular MCYSTs, the solid-phase extraction was performed using polymeric sorbent cartridges (Strata X 33-μm polymeric sorbent; Phenomenex Inc.). Analysis of both intracellular and extracellular MCYSTs was carried out by high-performance liquid chromatography-diode array detector using a Phenomenex Luna C_{18} column (250 by 4.6 mm; 5 μm). MCYSTs were identified on the basis of their characteristic absorption spectra, and the sum for all MCYST variants detected was calculated (see reference 14 for further analytical details).

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

$$X_t = \frac{X_{\max}}{1 + \left(\frac{X_{\max}}{X_0} - 1\right) \cdot e^{-\mu \cdot t}}$$

where $X_{\max}$ is the maximum cell yield (capacity), μ 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).

RESULTS

MCYST production in relation to different C_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 $C_{i,i}$. The variable fluorescence as a measure of $C_{i,i}$ 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 $C_{i,i}$ deficiency in cells grown in K medium

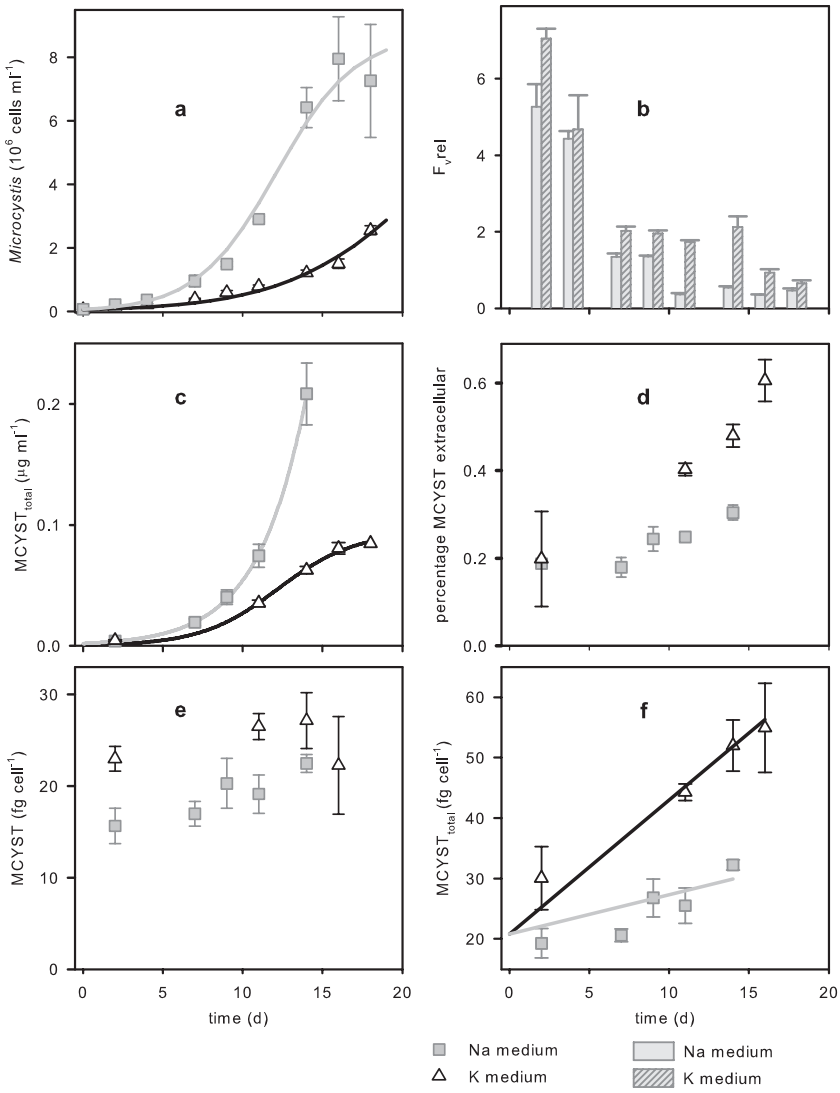

FIG. 1. Comparison of the effects of relatively high $C_{i,i}$ deficiency (K medium) and low $C_{i,i}$ deficiency (Na medium) on (a) growth, (c) volumetric MCYST concentrations, (e) MCYST cell quotas, (b) relative units of variable fluorescence ($F_{v,}$ rel), (d) the percentage of extracellular MCYSTs, and (f) the total MCYST production of *M. aeruginoa* PCC 7806 (growth parameters for Na medium, $\mu_{max} = 0.4$ day^{-1} and $X_{max} = 8.7 \times 10^6$ cells ml^{-1}; growth parameters for K medium, $\mu_{max} = 0.22$ day^{-1} and $X_{max} = 7.6 \times 10^6$ cells ml^{-1}). All symbols and bars indicate means, and standard errors of three replicates are indicated by error bars. See Table 1 for statistical tests of differences between treatments.

than in cells grown in Na medium (Table 1). Modification of the $C_{i,i}$ availability resulted in changes in key growth parameters. Both the maximum growth rate (μ_{max}) and X_{max} were reduced in K medium compared to Na medium (Fig. 1a). Cell sizes, however, were only slightly different for the two treatments (Table 1), thus allowing comparison of MCYST (and pigment) cell quotas. The culture volumetric MCYST concentrations for the two treatments nearly reflected the corresponding 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 finding of this experiment was the higher percentage of extracellular 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 elevated 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 respect 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 impact of MCYST on photosynthesis and/or growth, the effects of $C_{i,i}$ availability on cell number (yield), photosynthetic pigments, 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

TABLE 1. Effects of relatively low deficiency (Na medium) and high deficiency (K medium) of $C_{i,i}$ on different MCYST values, pigment content, relative units of variable fluorescence, and cell size of *M. aeruginosa* PCC 7808 in batch experiment 1 ($n = 3$)[a]

Medium	MCYST cell quota (fg cell^{-1}), only intracellular[b]	MCYST production (fg cell^{-1} day^{-1}), intracellular + extracellular[c]	MCYST-LR/[D-Asp^3]-MCYST-LR ratio[b]	% of MCYST extracellular[b]	Chl *a* cell quota (fg cell^{-1})[b]	Phycocyanin cell quota (pg cell^{-1})[b]	$F_{v,rel}$ (10^{-4} cell^{-1})[d]	Cell diam (μm)[b]
Na (high $C_{i,i}$)	18.0 (0.95)	0.65 (0.3)	0.25 (0.01)	0.21 (0.01)	25.7 (3.5)	1.20 (0.02)	2.55 (0.14)	2.35 (0.01)
K (low $C_{i,i}$)	23.7 (0.96)	2.2 (0.3)	0.31 (0.03)	0.47 (0.03)	29.2 (1.6)	2.46 (0.16)	2.64 (0.15)	2.45 (0.04)
P value	0.01[e]	0.0001[e]	0.08	0.0007[e]	0.15	0.001[e]	0.049[e]	0.065

[a] 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.
[b] Means for exponential growth were tested.
[c] A linear mixed model (38) 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.
[d] $F_{v,rel}$, relative units of variable fluorescence. Data were fitted by using $F_{v,rel} = a \cdot e^{-b \cdot t}$ and parameter *b* was tested.
[e] Significant at $\alpha = 0.05$.

proxy for the $C_{i,i}$ status of the cells) indicates the different responses of the wild type and the mutant to different $C_{i,i}$ states. As anticipated by theory, the variable fluorescence reflected the $C_{i,i}$ 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 $C_{i,i}$ 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 phycocyanin content with a more pronounced $C_{i,i}$ deficiency (K medium) (Fig. 2d and Table 2). Moreover, the response of the wild type to $C_{i,i}$ deficiency was more explicit than the response of the mutant. However, no interaction effect was found (Table 2). Distinct differences in the responses of the two strains were observed for the cell yield and Chl *a* content (Table 2). While modification of the $C_{i,i}$ status of the cells had no influence on the cell yield of the wild type, the yield of the mutant was significantly lower in the low-$C_{i,i}$ K medium than in the high-$C_{i,i}$ Na medium (Fig. 2a and Table 2). In contrast to the mutant, the wild type showed a clearly elevated concentration of Chl *a* with more pronounced $C_{i,i}$ deficiency (K medium) (Fig. 2b).

Intracellular MCYSTs during a dark-light change. One may argue that limiting the $C_{i,i}$ 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 $C_{i,i}$ 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 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 $C_{i,i}$ and to strain (wild type and mutant) as a proxy for the ability or inability to produce MCYSTs

Parameter	Variable[a]	df	Mean squares	*F* value	*P* value
Microcystis concn (cells ml^{-1})	Strain	1	1.08×10^{10}	0.05	0.84
	Medium	1	1.13×10^{12}	4.73	0.06
	Strain × medium	1	2.24×10^{12}	9.37	0.02
	Residuals	8	2.39×10^{11}		
Chl *a* concn (fg cell^{-1})	Strain	1	7.47×10^{1}	4.89	0.06
	Medium	1	1.32×10^{3}	86.64	1.0×10^{-5}
	Strain × medium	1	5.15×10^{2}	33.75	4.0×10^{-4}
	Residuals	8	1.53×10^{1}		
Phycocyanin concn (fg cell^{-1})	Strain	1	2.11×10^{5}	3.12	0.12
	Medium	1	4.15×10^{5}	6.14	0.04
	Strain × medium	1	1.25×10^{5}	1.85	0.21
	Residuals	8	6.76×10^{4}		
Relative units of variable fluorescence related to equal cell concn	Strain	1	6.58×10^{-11}	1.97	0.20
	Medium	1	1.85×10^{-9}	55.38	1.0×10^{-4}
	Strain × medium	1	3.68×10^{-13}	0.01	0.92
	Residuals	8	3.35×10^{-11}		

[a] All variables were measured in the exponential growth phase.

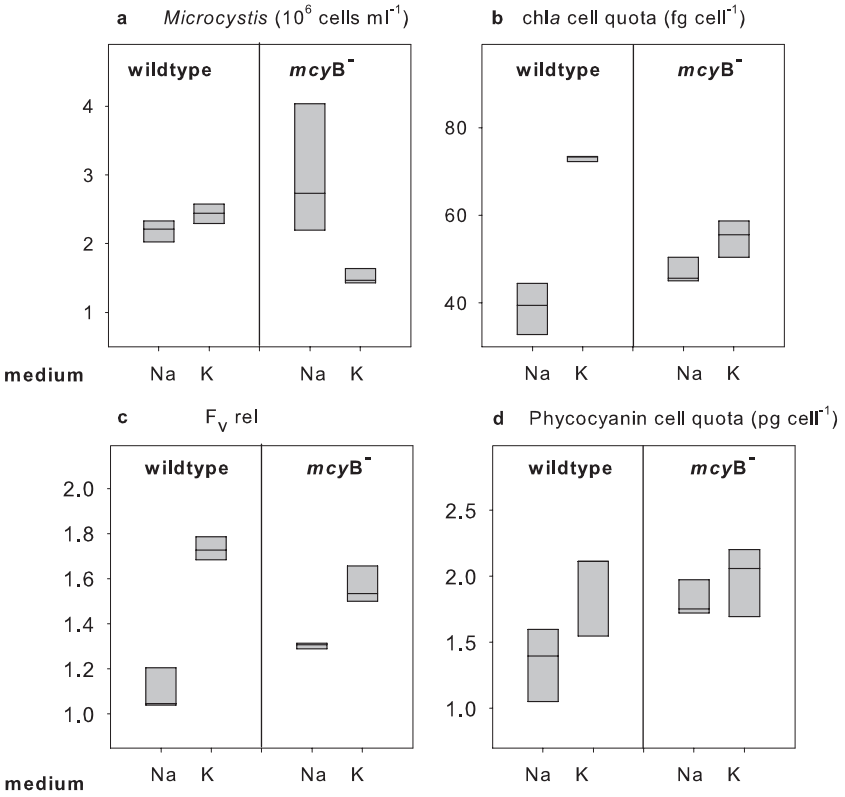

FIG. 2. Comparison of the effects of relatively low $C_{i,i}$ deficiency (Na medium) and high $C_{i,i}$ deficiency (K medium) on (a) cell yield, (b) Chl *a* cell quota, (c) relative units of variable fluorescence ($F_{v,}$ rel), and (d) phycocyanin cell quota of the MCYST-producing wild-type strain *M. aeruginosa* PCC 7806 and its *mcyB* mutant. The box plots show the results for three replicates at the exponential growth phase (batch cultures).

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 $C_{i,i}$ 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 $C_{i,i}$ conditions have an influence on MCYST production. In freshwater cyanobacteria, intracellular accumulation of C_i by the CCM is induced primarily by depletion of the $C_{i,i}$ pool (55). Photorespiratory metabolites have also been discussed as further elicitors (18, 55). We adjusted the cellular status for relative low or high $C_{i,i}$ 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 $C_{i,i}$ 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 $C_{i,i}$ 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 $C_{i,i}$ deficiency irrespective of their ability to produce MCYSTs.

In all test designs, experimentally increased $C_{i,i}$ 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 $C_{i,i}$ deficiency and MCYSTs cannot answer this question, as both intra- and extracellular levels of MCYSTs were enhanced under $C_{i,i}$-deficient conditions. Based on previous investigations, the occurrence of extracellular MCYSTs is ascribed to culture density or

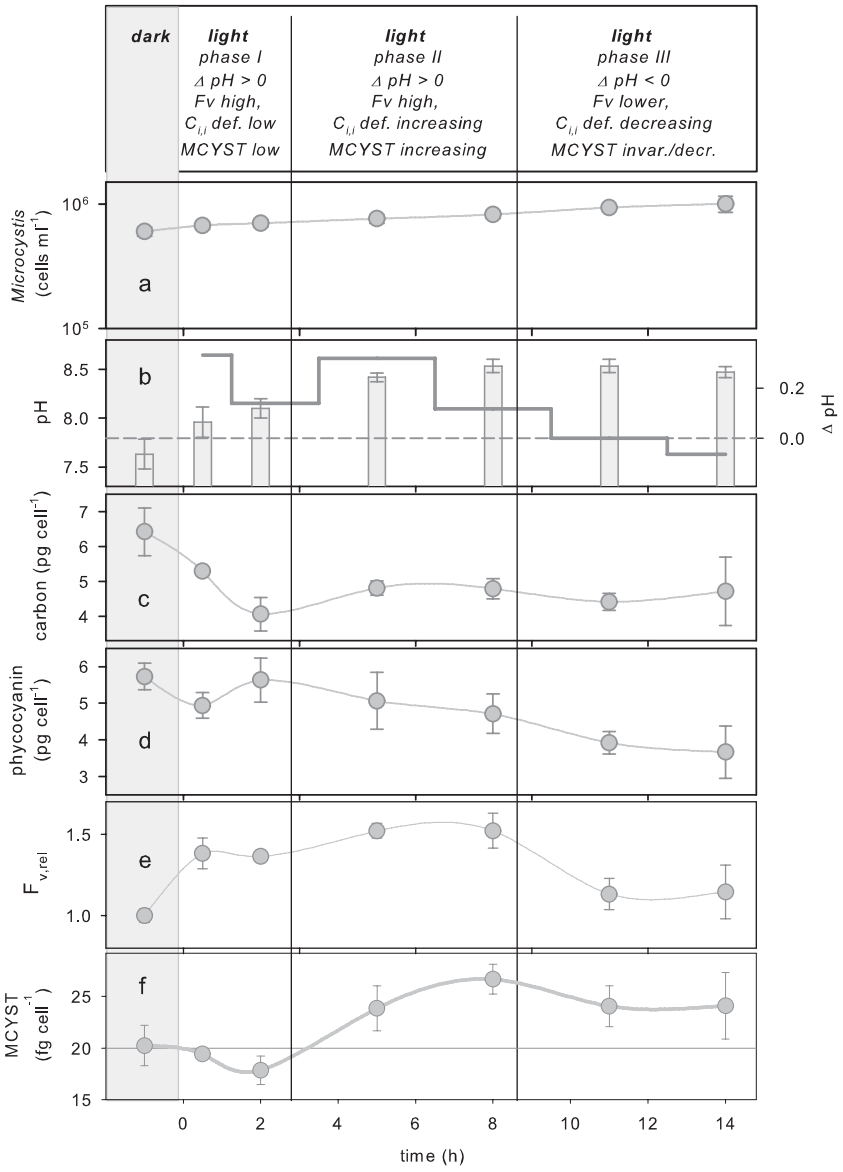

FIG. 3. Dark-light changes in a growing *M. aeruginosa* PCC 7806 batch culture, including (a) cell concentration (note the logarithmic scale), (b) pH value and pH change (Δ pH) between two consecutive sampling points, (c) total carbon concentration, (d) phycocyanin cell quota, (e) relative units of the variable fluorescence ($F_{v,rel}$), and (f) MCYST cell quota. All values are means ± standard errors obtained from three replicates. *Fv*, variable fluorescence; *def.*, deficiency; *invar./decr.*, invariate/decreasing.

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 *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 $C_{i,i}$ 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 $C_{i,i}$ accumulation, all these findings may be interpreted as an effect of relative $C_{i,i}$ 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-

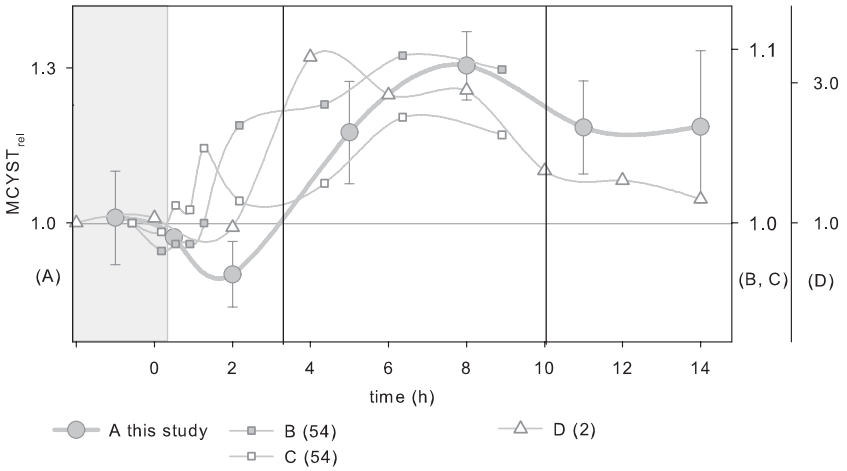

FIG. 4. Synopsis of changes in the MCYST cell quotas in response to diurnal dark-light change determined by different authors. Data were obtained in this study (gray circles), by using continuous cultures of *M. aeruginosa* PCC 7806 at light intensities of 10 and 40 μmol m^{-2} s^{-1} (gray and open squares, respectively) (54), and by using a batch culture of *M. panniformis* at a light intensity of 75 μmol m^{-2} s^{-1} (open triangles) (2). Relative MCYST cell quotas ($MCYST_{rel}$) were calculated from the original data by determining the ratio of the MCYST cell quota at any sampling time to the MCYST cell quota at the end of the dark phase.

est MCYST content is in the thylakoid area (56). Therefore, it also appears to be likely that MCYSTs may be involved in processes associated with the light reactions of photosynthesis. Regarding a putative specific function for MCYSTs inside the cyanobacterial cell, both implications—the involvement in controlling RuBisCO and the light reactions of photosynthesis—correspond fairly well with our results.

The dynamics of the variable fluorescence and cell phycocyanin quotas as indicators of the $C_{i,i}$ status of the cells showed that in subphase III of the third experiment distinct $C_{i,i}$ accumulation must have occurred. This provided the opportunity to compare two phases with low $C_{i,i}$ deficiency, subphases I and III. However, only in subphase III was the low $C_{i,i}$ deficiency caused by elevated $C_{i,i}$ accumulation. Unlike the low $C_{i,i}$ deficiency in subphase III, in subphase I the $C_{i,i}$ deficiency was low due to the transition from the dark phase to the light phase. However, cells became increasingly $C_{i,i}$ deficient due to the proceeding carboxylation. We assumed that the cells sensed the increasing $C_{i,i}$ deficiency during the transition from subphase I to subphase II and that this triggered MCYST production. As a result, $C_{i,i}$ 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 regimens and cell densities, which may have generated different states of $C_{i,i}$ deficiency.

Chl *a* quotas reacted differently to increased relative $C_{i,i}$ 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 $C_{i,i}$ 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 $C_{i,i}$ 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 C_i conditions by adjusting the phycocyanin/Chl *a* ratio (10, 26). This adaptation to the prevailing $C_{i,i}$ conditions facilitates energization of C_i uptake (21, 27) and $C_{i,i}$ 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 $C_{i,i}$ deficiency compared to $C_{i,i}$-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 $C_{i,i}$ 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 $C_{i,i}$ deficiency even in the wild-type strain could not be fully compensated for to the same extent as under $C_{i,i}$-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 $C_{i,i}$ variants. Since there was close agreement in all experiments between the ability to adjust the photosynthetic pigments to the $C_{i,i}$ status and the extent of MCYST production, we concluded that MCYSTs might be involved directly or indirectly in $C_{i,i}$ accumulation. Furthermore, based on the time pattern seen in experiment 3 it is proposed that MCYST production precedes $C_{i,i}$ accumulation. As the ratio of the two MCYST variants (MCYST-LR and [D-Asp^3]-MCYST-LR) produced by strain PCC 7806 was not influenced by the $C_{i,i}$ 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 nonproducing 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 $C_{i,i}$ conditions is one of the few existing explanations of the ecophysiological 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 $C_{i,i}$ deficiency meet this requirement. A relative physiological $C_{i,i}$ 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 cyanobacteria having the ability to keep the internal CO_2/O_2 ratio high by an effective CCM, in which MCYSTs might be involved. However, further investigations beyond the scope of this study are necessary to test this hypothesis on the molecular level.

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