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A Mutant Isolated from the Cyanobacterium *Synechococcus* PCC7942 Is Unable to Adapt to Low Inorganic Carbon Conditions[1]

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Using a novel screening procedure, we have selected a new class of mutant from the cyanobacterium *Synechococcus* PCC7942 that fails to adapt to growth at an extremely low inorganic carbon (C_i) concentration. The mutant (Tm17) reported in this study grows normally at or above air levels of CO_2 (340 μL L^{-1}) but does not survive at 20 μL L^{-1} CO_2 in air. Air-grown Tm17 cells showed properties similar to wild-type cells in various aspects of the CO_2-concentrating mechanism examined. Following transfer from air levels to 20 μL L^{-1} CO_2, however, the mutant cells failed to increase their photosynthetic affinity for C_i. This results in an approximately 10-fold difference in photosynthetic affinity between the wild-type and Tm17 cells under C_i-limiting conditions [the $K_{0.5}(C_i)$ values were 11 and 136 μM, respectively]. Further examination of factors possibly contributing to this low photosynthetic affinity showed that Tm17 cells have no inducible high-affinity HCO_3^- transport and do not appear to show induction of increased carboxysomal carbonic anhydrase and ribulose-1,5-bisphosphate carboxylase/oxygenase activities. It appears that a common factor, possibly relating to CO_2 detection and/or induction signal, or the HCO_3^- transport mechanism may have been impaired in the mutant. Complementation results indicate that the mutation responsible for the phenotype has occurred in an 8- to 10-kb *Eco*RI genomic DNA fragment.

Elevation of CO_2 by the novel CCM in cyanobacteria greatly increases the efficiency of CO_2 fixation in these organisms. Research in the past decade has demonstrated that a functional CCM in cyanobacteria comprises two parts: (a) a C_i transport system that actively accumulates C_i from the surrounding medium and (b) the carboxysome that provides a compartment for the localized elevation of CO_2 concentration around Rubisco (Badger and Price, 1992). Although the mechanics of the C_i transport system are not fully understood, evidence suggests that a "CA-like" component is involved (Volokita et al., 1984; Price and Badger, 1989a; Espie et al., 1991; Miller et al., 1991; Badger and Price, 1992). The transport system can utilize both CO_2 and HCO_3^- as substrates (Price and Badger, 1989a) but delivers HCO_3^- as the C_i species into the cytoplasm of the cell (Volokita et al., 1984; Price and Badger, 1989c). Catalyzed dehydration of HCO_3^- apparently occurs only in the carboxysomes where CA activity is specifically localized (Price et al., 1992). The corresponding gene for carboxysomal CA has been isolated (Fukuzawa et al., 1992; Yu et al., 1992). Because of the special properties of the carboxysome, wasteful CO_2 leakage is minimized and its concentration is thus elevated in the vicinity of the CO_2-fixing enzyme, Rubisco.

The efficiency of the CCM changes in response to the environmental conditions, especially the availability of C_i. When cyanobacterial cells that have been grown in a relatively high C_i (e.g. 340 μL L^{-1}) are transferred to lower C_i conditions, a higher photosynthetic affinity for C_i is observed (Badger and Andrews, 1982; Mayo et al., 1986; Badger and Gallagher, 1987). Correlating with this increase in photosynthetic efficiency are the induction of a higher affinity C_i transport system and an increase in the number of carboxysomes (Turpin et al., 1984) and the activity of carboxysomal CA (Price et al., 1992). Although the processes involved in induction are poorly understood, it is clear that induction involves protein synthesis and is light dependent (Marcus et al., 1982).

Selection and characterization of mutants have proven to be powerful tools for studies of the CCM in cyanobacteria. The study described here is part of our ongoing efforts to recover mutants defective in various components of the process. The novel selection procedure was originally designed to screen for mutations in HCO_3^- transport that may include the primary transport proteins and the pathways for energization of the transport process (Badger et al., 1991). The mutants obtained thus far in *Synechococcus* PCC7942 were selected for growth at elevated CO_2 (1–5%) but with an inability to grow at air levels of CO_2. Most are defective in the ability to use the internal C_i pool (Badger et al., 1991). The only reported mutants with defects in C_i transport have been isolated from *Synechocystis* PCC6803 and have lesions

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Abbreviations: CA, carbonic anhydrase; CCM, CO_2-concentrating mechanism; C_i, dissolved inorganic carbon; EZ, ethoxyzolamide; I_{50}, inhibitor concentration required for half-maximal inhibition; $K_{0.5}(C_i)$, $K_{0.5}(CO_2)$, and $K_{0.5}(HCO_3^-)$, concentration of total inorganic carbon, CO_2, and HCO_3^-, respectively, required for half-maximal response; TTES, BG medium containing 1% agar and 50 mM Tes (pH 8.0).

in a thylakoid-located NADH dehydrogenase gene cluster that is probably involved in energization of C_i transport (Ogawa, 1992a, 1992b). Using the model of Reinhold et al. (1989), we predicted that a mutation in the HCO_3^--utilizing system, either for a one- or a two-pump model, would produce a mutant with a medium to high C_i requirement for growth (Badger et al., 1991). Air would be a permissive CO_2 condition and 20 μL L^{-1} would be a nonpermissive CO_2 level. Using these conditions for mutagenesis and selection, we have obtained a new class of mutants of *Synechococcus* PCC7942. The mutant reported here, Tm17, appears to have normal photosynthetic physiology when grown at air levels of CO_2, but it was unable to survive after transfer to C_i-limiting conditions, i.e. 20 μL L^{-1} CO_2 in air.

MATERIALS AND METHODS

Growth Conditions

Liquid cultures of *Synechococcus* PCC7942 (formerly known as *Anacystis nidulans* R2) were grown under conditions similar to ones previously described (Price and Badger, 1989a). CO_2 concentrations in the gas phase were 2% (v/v), or air level (about 340 μL L^{-1}), or 20 μL L^{-1}, as indicated. Unless otherwise stated, the light intensity was 100 μmol of photons m^{-2} s^{-1}. Cells were harvested by centrifugation at room temperature when the Chl density reached about 3 μg mL^{-1}. Plate colonies were grown on 1% agar/BG11 medium (Rippka et al., 1981) buffered with 50 mM Tes-KOH (TTES plates) in transparent plastic boxes as described previously (Price and Badger, 1989b).

Mutagenesis and Selection of Mutants

The procedure was modified from the protocol used for selection of high CO_2-requiring mutants as described by Price and Badger (1989d). Actively growing *Synechococcus* PCC7942 cells (2 ml; 10^9 cells mL^{-1}) were incubated with 0.2 M ethylmethyl sulfonate in 30 mM phosphate buffer (pH 7.0) in the dark for 45 min at 37°C. The mutagen was then inactivated with 5% sodium thiosulfate (pH 8.0). The cells were washed twice in unbuffered BG11 medium and diluted to 10^{-2}, 10^{-3}, and 10^{-4} of the starting density. A 50-μL aliquot of diluted cells was plated onto a cellulose disc (7.5 cm in diameter) laid on top of a TTES plate so that colonies could be transferred from one plate to another without perturbation. Each colony represents an independent mutation. Two plates of each dilution were set up. Care was taken to minimize water condensation on the cellulose disc during growth.

Plates were incubated at permissive CO_2 conditions (air levels) and low light (10 μmol of photons m^{-2} s^{-1}) for 21 h and then transferred to nonpermissive CO_2 conditions (20 μL L^{-1}) for 2 d to deplete internal carbon reserves. An ampicillin-enrichment step was then imposed by transferring the cellulose discs with the cells on top to plates containing 100 μg mL^{-1} of ampicillin and incubating them at 20 μL L^{-1} of CO_2 and normal light intensity (30 μmol of photons m^{-2} s^{-1}) for 19 h. The cellulose discs were then transferred back to normal TTES plates and incubated at 20 μL L^{-1} of CO_2 for another 6 d. Wild-type colonies were marked, and the plates were returned to air. Putative mutants appeared 3 d later, and a total of 103 putative mutants were collected. After rescreening on duplicate plates under nonpermissive and permissive CO_2 conditions, six were obtained with various degrees of inability to grow at 20 μL L^{-1} of CO_2 compared to wild-type. One of them, designated Tm17, failed to grow on 20 μL L^{-1} of CO_2 plates and was examined in this study.

Measurement of CO_2 and HCO_3^- Uptake during Steady-State Photosynthesis

Activity of CO_2 and HCO_3^- uptake during steady-state photosynthesis was measured in a glass cuvette connected to a mass spectrometer as described by Badger et al. (1993). Cells were washed twice with the assay buffer aerated with CO_2-free air. The Chl content in the assay medium was 3 to 5 μg mL^{-1}. O_2 evolution and CO_2 uptake in the light were measured simultaneously in the closed cuvette at a light intensity of 300 μmol of photons m^{-2} s^{-1} and at a temperature of 30°C. The assay medium was BG11 medium (Rippka et al., 1981) buffered with 50 mM 1,3-bis[tris(hydroxymethyl)methylamino]propane (pH 8.0). The light was turned off after steady-state rates of photosynthetic O_2 evolution and CO_2 uptake were recorded, and the initial CO_2 efflux in the dark was measured. Subsequent addition of C_i was made after CO_2 reached equilibrium with HCO_3^- in the assay medium.

Complementation by Wild-Type Genomic DNA

Wild-type chromosomal DNA of *Synechococcus* PCC7942 was prepared according to the protocol of Dzelzkalns et al. (1984) and digested to completion with an appropriate restriction enzyme. When required, restriction fragments were fractionated by size in a preparative electrophoresis device (ELFE, Genofit, Switzerland) as described by Price and Badger (1989c). Actively growing cells in liquid culture were harvested and washed in unbuffered BG11 medium. An aliquot of a 50-μL cell suspension (containing about 10^8 cells) was incubated with about 0.5 μg of DNA fragments under low light (<10 μmol of photons m^{-2} s^{-1}) at 30°C for about 4 h. The cells were then spread on the TTES plates and grown for 16 to 24 h in air in the light (30 μmol of photons m^{-2} s^{-1}) before being transferred to 20 μL L^{-1} of CO_2 in air. Complemented colonies were scored after 10 d.

Measurement of CA and Rubisco Activity in Crude Extracts

Cells were lysozyme-treated and broken by French press treatment as described by Price et al. (1992). The homogenate was spun for 60 s in a bench-top microfuge to pellet unbroken cells, and crude carboxysome preparations were obtained from the homogenate after precipitation with 20 mM $MgSO_4$ as described by Yu et al. (1992). CA activity was determined by a mass spectrometric method and Rubisco by $NaH^{14}CO_3$ as previously described (Price et al., 1992).

Other Measurements

C_i accumulation time courses and photosynthetic O_2 evolution measurements in the O_2 electrode were performed as

previously described (Price and Badger 1989b). Chl was estimated in ethanol according to the procedure of Wintermans and de Mots (1965).

RESULTS

Affinity for C_i

When grown at various CO_2 concentrations, wild-type cells of *Synechococcus* PCC7942 have the ability to adapt to the growth C_i conditions by changing their affinity for C_i so that a high level of photosynthetic CO_2 fixation is maintained (Fig. 1). When the cells were grown at 2% CO_2, about 2 mM C_i was required to reach maximal photosynthetic O_2 evolution. As growth C_i decreased to air levels or following induction at 20 μL L^{-1} for 16 h, much less C_i (0.7 and 0.1 mM, respectively) was required to saturate photosynthesis. In contrast, mutant Tm17 did not show such a dramatic adaptation to the changes in growth C_i concentrations (Fig. 1).

Comparison of the $K_{0.5}(C_i)$ values estimated from these experiments clearly shows that wild-type cells increase their affinity for C_i when the growth CO_2 concentration decreases; the greatest change occurs between air level and 20 μL L^{-1} of CO_2 with an order of magnitude decrease in the $K_{0.5}(C_i)$ value (Table I). Similar values were obtained for mutant Tm17 when the growth C_i was at or above air levels (Table I). However, when the growth C_i was further decreased to 20 μL L^{-1}, the mutant failed to show a significant increase in its photosynthetic affinity for C_i (Table I). This results in an approximately 10-fold difference between the wild-type (11 μM) and Tm17 cells (136 μM) in their photosynthetic $K_{0.5}(C_i)$ values under C_i-limiting conditions. In fact, the mutant cells showed signs of photoinhibition after about 16 h of induction, as indicated by the reduction in the V_{max} value (Table I, Fig. 1). Prolonged exposure to such C_i conditions leads to the death of the mutant, probably as a result of an inability of the mutant to induce a high-affinity CCM under C_i-limiting conditions.

Table I. *Changes in the kinetic parameters of photosynthesis in wild-type and Tm17 cells grown at different CO_2 concentrations*

The data are means ± SD from at least three measurements in the mass spectrometer at 30°C and at 300 μmol of photons m^{-2} s^{-1} light and assayed in BG11 medium buffered with 50 mM 1,3-bis[tris(hydroxymethyl)methylamino]propane (pH 8.0).

Cells	Growth [CO_2]	$K_{0.5}$ (C_i)	V_{max}
		μM	μmol mg^{-1} of Chl h^{-1}
Wild type	2% (v/v)	240 ± 76	241 ± 34
	Air (350 mL L^{-1})	203 ± 56	461 ± 68
	20 μL L^{-1}	11 ± 6	317 ± 29
Tm17	2% (v/v)	313 ± 35	297 ± 35
	Air (340 μL L^{-1})	165 ± 57	370 ± 75
	20 μL L^{-1}	136 ± 31	255 ± 27

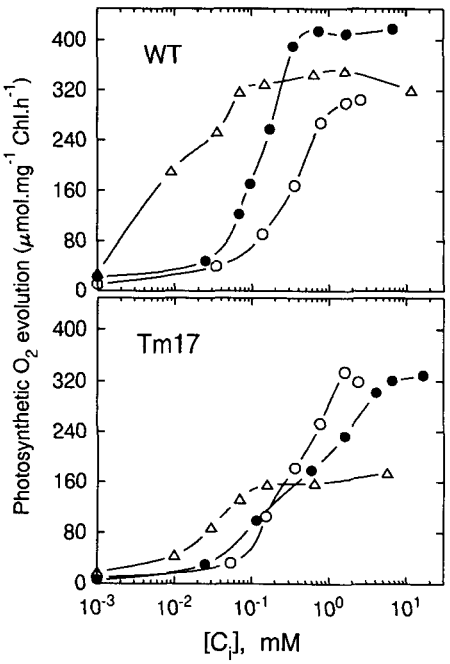

Figure 1. The response of photosynthetic O_2 evolution to inorganic carbon in wild-type (WT) and Tm17 cells of *Synechococcus* PCC7942. Cells were grown at 2% CO_2 in air (○), or air levels of CO_2 (●), or air levels of CO_2 but induced at 20 μL L^{-} of CO_2 for 24 h before measurements (△) under continuous illumination of 100 μmol of photons m^{-2} s^{-1}. Measurements were made at 30°C in BG11 medium (pH 8.0) with a light intensity of 300 μmol of photons m^{-2} s^{-1}.

Induction Time Courses

A time-course experiment was carried out to identify when air-grown Tm17 cells become different from the wild-type during adaptation to limited C_i conditions (Fig. 2). Estimation of the $K_{0.5}(C_i)$ values in photosynthetic O_2 evolution revealed that wild-type cells had two phases during the adaptation to limited C_i: a rapid initial phase, followed by a slow phase (Fig. 2a). During the rapid phase, a significant decrease in $K_{0.5}(C_i)$ occurred within 1.5 h of the start of induction, and this rapid phase lasted for 4 h, during which the $K_{0.5}(C_i)$ decreased more than 3-fold. This was followed by a slower decline phase, during which the $K_{0.5}(C_i)$ decreased from 243 μM in air-grown cells to less than 10 μM after a 24-h induction at 20 μL L^{-1} of CO_2. A substantial difference was observed within 4 h of induction between wild-type and Tm17 cells. Although Tm17 cells showed some reduction in their photosynthetic $K_{0.5}(C_i)$ values during the induction, the values remained greater than 120 μM after 24 h of induction (Fig. 2a). In contrast to the dramatic change in the $K_{0.5}(C_i)$ values, however, the maximal photosynthetic capacity (V_{max}) of wild-type cells showed much less change throughout the experiment (Fig. 2b), and somewhat greater reduction in V_{max} was observed in Tm17 cells after 24 h at 20 μL L^{-1} of CO_2 (about half of the starting value, Fig. 2b).

Thus, unlike other high CO_2-requiring mutants (Badger et al., 1991), photosynthesis in mutant Tm17 is not significantly different from wild-type cells when they were grown at or above the air levels of CO_2 concentrations (Figs. 1 and 2, Table I). This would explain the similarity between them in growth at 2% CO_2 in air or air levels of CO_2 (Fig. 3). Both

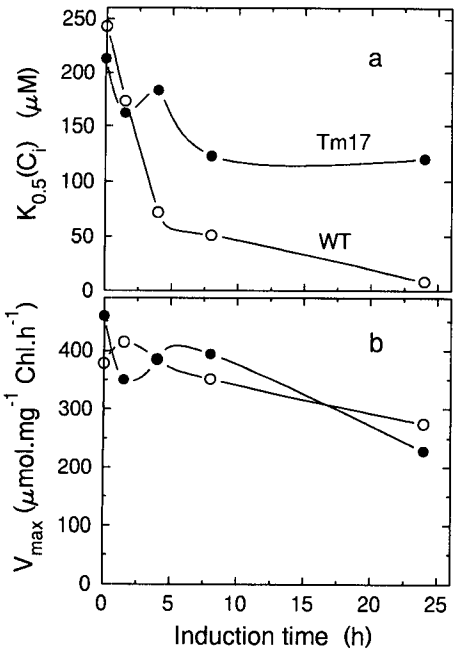

Figure 2. A time course of induction of photosynthetic O_2 evolution in wild-type (WT) and Tm17 cells of *Synechococcus* PCC7942. Both wild-type (O) and the mutant (●) cells were grown at air levels of CO_2 and transferred to 20 μL L^{-1} at time zero. At the indicated time intervals, aliquots were taken for measurement of photosynthetic C_i response under the same conditions as in Figure 1. The values of $K_{0.5}(C_i)$ and V_{max} were estimated from such responses.

grew better at the higher CO_2 concentration. After 9 d of growth, the cell density reached about 3×10^8 cells mL^{-1} at air and 5×10^8 cells mL^{-1} at 2% CO_2. Both appeared to be able to continue to grow beyond the time indicated under the conditions used, especially when a high CO_2 concentration was available.

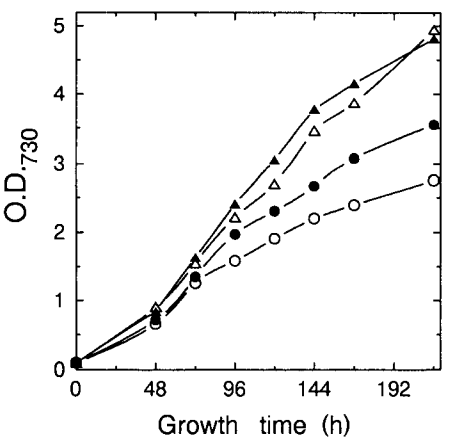

Figure 3. A time course of growth of wild-type (open symbols) and Tm17 cells (closed symbols) of *Synechococcus* PCC7942. Actively growing cells were inoculated to a density of 0.1 A_{730} unit. Growth at air levels of CO_2 (circles) or 2% CO_2 in air (triangles) was followed by measuring the A_{730}.

A higher internal C_i pool is normally observed in high CO_2-requiring mutants with defects in the ability to utilize accumulated C_i for CO_2 fixation (Price and Badger, 1989d). However, when the C_i pool was measured in Tm17 cells, little difference was found relative to wild-type cells (Table II). Fully induced cells were able to accumulate nearly double the amount of C_i of that in air-grown cells. In wild-type cells, this larger C_i pool must have been accumulated by the induction of high-affinity transport of both CO_2 and HCO_3^-. In Tm17 cells, however, it is probably accumulated primarily by the CO_2 transport system in the absence of high-affinity HCO_3^- transport (see below).

Rubisco and CA Activity

CA and Rubisco are two important enzymes that affect the photosynthetic performance of cyanobacteria (Badger and Price, 1992). With improved procedures, two types of CA activity have been identified in cyanobacteria and can be distinguished by their sensitivity to inhibition by EZ (Price et al., 1992). The CA activity associated with purified carboxysomes has an I_{50} value for EZ inhibition of 4 μM (Price et al., 1992), and the enzyme expressed in *Escherichia coli* had an I_{50} of 2 μM (Yu et al., 1992). Carboxysomal CA activity was totally inhibited with 30 μM EZ. The other CA activity was much less sensitive to inhibition by EZ with a much higher I_{50}(EZ) of about 150 μM (Price et al., 1992). Therefore, the low-I_{50} (carboxysomal) and high-I_{50} (possibly membrane bound in the C_i pump) CA activity can be measured separately, even in crude cell extracts, by the addition of 30 μM EZ.

The results of CA and Rubisco activities measured in crude extracts of wild-type and Tm17 cells are given in Table III. In wild-type cells, when the growth CO_2 concentration decreased from air levels to 20 μL L$^-$, Rubisco activity remained constant, whereas CA activity, including both low- and high-I_{50} components, increased. This resulted in a significant increase in the ratio of CA to Rubisco (Table III; Price et al., 1992). A similar increase in this ratio was also observed in Tm17 cells, but it was due to the reduction in Rubisco activity rather than an increase in the CA activity, as in the wild-type cells. Although air-grown Tm17 cells showed CA activities

Table II. *The internal C_i pool in wild-type and Tm17 cells*

Cells were grown in liquid media at air level CO_2 or induced at 20 μL L^{-1} of CO_2 for 11 h. Measurements were made with 30 μg mL^{-1} of Chl in the presence of 1 mM $NaHCO_3$ and 20 mM glycoaldehyde to inhibit CO_2 fixation under a light intensity of 300 μmol of photons m^{-2} s^{-1}. CA was added to the medium, and CO_2 concentrations were monitored by a mass spectrometer. When steady state was reached, light was turned off, and the amount of C_i evolved was taken as the size of the internal pool.

Cells	Growth [C_i]	Internal C_i Pool
		mM
Wild type	Air	27.6
	20 μL L^{-1}	46.3
Tm17	Air	23.2
	20 μL L^{-1}	40.6

comparable to wild-type cells, virtually no increase was obtained when induced at 20 μL L^{-1} of CO_2. It is not clear whether the reduction in Rubisco activity was a positive adaptation to achieve a higher ratio of CA to Rubisco or a consequence of inhibition occurring under the experimental conditions, but it is unlikely that lower activities of these enzymes alone, in comparison to wild-type cells, could have contributed to the low photosynthetic efficiency observed (Figs. 1 and 2; Table I).

Uptake of CO_2 and HCO_3^- Before and After Induction

The C_i transport system is another essential component that determines the photosynthetic performance of cyanobacteria. We have developed a mass spectrometric disequilibrium technique to measure the uptake activity of both CO_2 and HCO_3^- during steady-state photosynthesis (Badger et al., 1993). Compared to techniques used under non-steady-state conditions, such as the silicone oil centrifugation technique, this method provides a better estimation of C_i uptake and its interactions with other associated processes in the light. CO_2 and HCO_3^- uptake activities can be determined separately and $K_{0.5}(CO_2)$, $K_{0.5}(HCO_3^-)$, and V_{max} values can be estimated from respective substrate responses during steady-state photosynthesis. Figure 4 shows that there was no significant difference in CO_2 uptake between wild-type and Tm17 cells (Fig. 4, a and b). Both cells exhibited a stepwise increase in their affinity for CO_2 [decrease in $K_{0.5}(CO_2)$ values] as growth C_i decreased from 2% to 20 μL L^{-1} of CO_2, with V_{max} ranging from 100 to 200 μmol mg^{-1} of Chl h^{-1}. In contrast to CO_2 uptake, however, HCO_3^- uptake in the mutant showed no significant decrease in the $K_{0.5}(HCO_3^-)$ values when growth C_i was reduced from air levels to 20 μL L^{-1} of CO_2 for 8 h, although partial induction was observed between cells grown at 2% and air CO_2 levels (Fig. 4c). The $K_{0.5}(HCO_3^-)$ value obtained for fully induced cells was 117 μM in Tm17 cells, which was almost an order of magnitude higher than the value in wild-type cells of 15 μM. In other words, wild-type cells are able to adapt to the C_i-limiting conditions by increasing the affinity of its C_i transport system for both CO_2 and HCO_3^-, but Tm17 cells were only able to do so for CO_2 but not for HCO_3^-. Nevertheless, the maximal capacity for HCO_3^- transport in the mutant still reached a comparable level in the presence of higher substrate concentrations (Fig. 4d), saturating at 270 μM HCO_3^- instead of 50 μM in wild-type cells (data not shown).

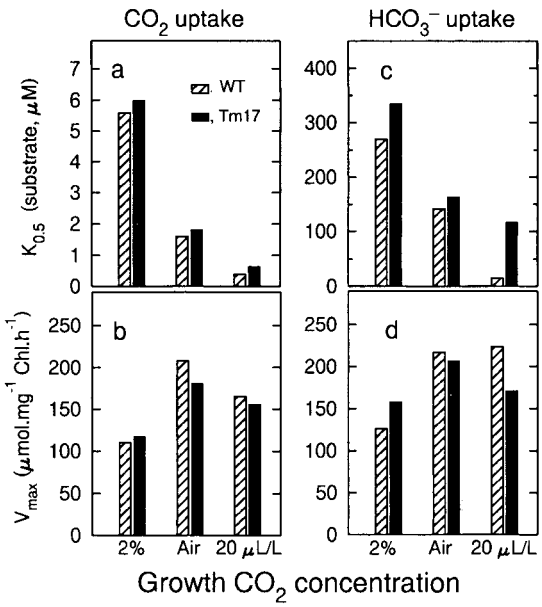

Figure 4. Changes in the kinetic parameters of CO_2 and HCO_3^- uptake in wild-type (WT) and Tm17 cells of *Synechococcus* PCC7942 grown at different CO_2 concentrations. The values were estimated from substrate responses of CO_2 uptake (a and b) and HCO_3^- uptake (c and d) and represent the means of at least three measurements.

Complementation with Genomic DNA Fragments

It appears that the low photosynthetic efficiency of Tm17 cells in C_i-limiting conditions is associated with defects in a number of physiological functions. To determine whether it is caused by multiple mutations or by a single mutation that affects the expression of all the other components, we attempted to isolate the complementary DNA fragment(s). The "dot" transformation procedure successfully used for complementation of type II mutants (Yu et al., 1992) did not work for Tm17 cells (not shown), probably because of the difficulty of CO_2 penetration into the top agarose. Complementation

Table III. *Rubisco and CA activity in crude extracts of wild-type and mutant TM17 cells*

Cells were grown at air level CO_2 and 100 μmol of photons m^{-2} s^{-1} light to a Chl content of about 3 μg mL^{-1}. Half of the culture was then induced at 20 μL L^{-1} of CO_2 for 18 h with 40 μmol of photons m^{-2} s^{-1} (to minimize photoinhibition). Low-I_{50} CA activity was that which is inhibited by 30 μM EZ and the remaining activity was regarded as high-I_{50} activity (Price et al., 1992; Yu et al., 1992). The data are the means of four measurements.

Cells	Growth [CO_2]	Rubisco	CA			Ratio of Low-I_{50} CA to Rubisco
			Total	High I_{50}	Low I_{50}	
		μmol mg^{-1} of Chl min^{-1}	units mg^{-1} of Chl			
Wild type	Air (340 μL L^{-1})	1.52	11.0	2.3	8.7	5.7
	20 μL L^{-1}	1.47	18.2	4.8	13.4	9.1
Tm17	Air (340 μL L^{-1})	1.38	10.0	2.7	7.3	5.3
	20 μL L^{-1}	0.87	10.9	2.9	8.0	9.2

was observed when mutant cells were first incubated with wild-type genomic DNA and then spread on normal TTES plates. The efficiency of transformation varied greatly with restriction fragments generated with different restriction endonucleases (data not shown). DNA cut with the restriction enzyme *Eco*RI gave high transformation efficiency. Complementation with fractionated *Eco*RI fragments indicated that the complementary fragment has a size in the range of 8 to 10 kb. In an attempt to clone the complementing gene, the DNA fragments in the fraction enriched with the complementary fragment were inserted into the pUC18 plasmid and propagated in the *E. coli* strain JM109. However, no complementation was observed with more than 700 individual clones tested. Using the same procedure (with 0.5 μg of plasmid DNA), we have also tried to complement Tm17, without success, with an 8.5-kb clone containing *rbcL* and a number of carboxysomal genes (Price and Badger, 1991) and the 3.5-kb pT2 clone containing the carboxysomal CA gene (Yu et al., 1992). Other approaches are being explored in an attempt to identify the mutation and the gene product responsible for the phenotype observed.

DISCUSSION

Screening with air as a permissive CO_2 concentration and 20 μL L^{-1} as a nonpermissive CO_2 concentration, we have obtained a new class of chemically induced mutants from *Synechococcus* PCC7942. The Tm17 mutant presented in this article showed little difference from the wild-type cells when the growth C_i was at or above air levels of CO_2, with respect to photosynthetic performance (Figs. 1 and 2, Table I), growth (Fig. 3), internal C_i pool sizes (Table II), CA and Rubisco activities (Table III), and C_i transport (Fig. 4). It is only when the cells were induced at very low C_i (20 μL L^{-1} of CO_2 in air) that significant differences were observed. In Tm17 cells, all parameters examined, except CO_2 transport and internal C_i pool sizes, failed to induce when the cells were transferred from air to 20 μL L^{-1} of CO_2. In fact, the mutant was not able to survive prolonged incubation under severely C_i-limited conditions. Recently, Ogawa (1992b) reported the isolation of a *Synechocystis* PCC6803 mutant (SC) that also grows normally at air levels of CO_2 but is unable to grow at CO_2 concentrations less than 80 μL L^{-1}. In direct contrast to our mutant Tm17, however, mutant SC has a defect only in CO_2 transport but not in HCO_3^- transport. Nevertheless, the results from these two mutants indicate that CO_2 and HCO_3^- transport can be manipulated separately and that they are apparently mediated by separate systems. Unfortunately, the mutagenesis procedure for the isolation of mutant SC was not presented, which may be used in *Synechococcus* PCC7942.

It is reasonable to assume that carboxysomal CO_2 fixation and membrane C_i transport are the primary factors determining the photosynthetic efficiency of cyanobacteria (Badger and Price, 1992). In addition, Rubisco and CA are two of the primary enzymes involved in CO_2 fixation. Wild-type cells normally have higher activities of these enzymes when they adapt to low C_i conditions (Table III; Price et al., 1992). The lack of such an increase in Tm17 cells (Table III) could have been partly responsible for the low photosynthetic efficiency observed under C_i-limiting conditions (Figs. 1 and 2; Table I). Results of DNA complementation, however, indicated that the mutation responsible for the phenotype is not in the structural genes or upstream-flanking regions of these enzymes.

It is possible that a lower photosynthetic efficiency in Tm17 cells is a result of the lack of induction of the high-affinity HCO_3^- uptake mechanism (Fig. 4). The C_i transport system in wild-type cells, when adapted to 20 μL L^{-1} of CO_2 in air, has a very high affinity for HCO_3^-, with a $K_{0.5}(HCO_3^-)$ value of 10 μM and a maximal activity of 220 μmol of HCO_3^- mg^{-1} of Chl h^{-1} saturated at less than 0.1 mM HCO_3^- (Fig. 4). This high-affinity HCO_3^- uptake, however, was not detected in Tm17 cells (Fig. 4). There are two types of high-affinity HCO_3^- uptake, i.e. Na^+ dependent and Na^+ independent (Espie et al., 1991). Na^+-independent HCO_3^- transport becomes significant when cells are grown in standing culture or bubbling with 30 μL L^{-1} of CO_2 in air. If a defect in Na^+-independent HCO_3^- transport were responsible for the phenotype of Tm17 cells, then such transport must be an essential part of the C_i transport system in cyanobacteria during adaptation to an extremely low C_i environment. However, we have found that HCO_3^- transport is tightly feedback regulated by CO_2 fixation (our unpublished data). A low-affinity HCO_3^- uptake could be the result of a slightly impaired ability to fix CO_2 within the cell, as may be caused by the failure to induce carboxysomal CA (Table III).

Another way to explain the mutant phenotype in Tm17 is to postulate that the cells lack a single factor that involves detection and/or production of an inducing signal. This common factor might be required for expression of components that confer high photosynthetic efficiency on the cell, e.g. a higher carboxysomal CA activity (Table III; Price et al., 1992) and a high-affinity component(s) for C_i uptake. At present, very little is known about the way that cyanobacteria detect a decrease in CO_2 concentrations, the transduction pathways that lead to gene expression, and the regulatory mechanisms that control and coordinate the interaction between various components. Variation of the ratio of carboxylase to oxygenase activity of Rubisco on transfer to low C_i has been proposed to be involved in induction, and phosphoglycolate has been suggested to be a signal for adaptation to low CO_2 conditions (Marcus et al., 1983). Alternatively, the cells might be able to directly monitor the CO_2 concentration in the external medium, e.g. by a promoter that is activated by low CO_2 concentration (Scanlan et al., 1990). These possibilities require further analysis. Complementation of Tm17 by wild-type genomic DNA indicates that the mutation is located in an 8- to 10-kb *Eco*RI fragment. Cloning and analysis of this fragment, currently in progress, may shed some light on the complex mechanism of how cyanobacteria detect and adapt to low CO_2 concentrations.

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