Identification of an ATP-binding cassette transporter involved in bicarbonate uptake in the cyanobacterium *Synechococcus* sp. strain PCC 7942

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Exposure of cells of cyanobacteria (blue–green algae) grown under high-CO_2 conditions to inorganic C-limitation induces transcription of particular genes and expression of high-affinity CO_2 and HCO_3^- transport systems. Among the low-CO_2-inducible transcription units of *Synechococcus* sp. strain PCC 7942 is the *cmpABCD* operon, encoding an ATP-binding cassette transporter similar to the nitrate/nitrite transporter of the same cyanobacterium. A nitrogen-regulated promoter was used to selectively induce expression of the *cmpABCD* genes by growth of transgenic cells on nitrate under high CO_2 conditions. Measurements of the initial rate of HCO_3^- uptake after onset of light, and of the steady-state rate of HCO_3^- uptake in the light, showed that the controlled induction of the *cmp* genes resulted in selective expression of high-affinity HCO_3^- transport activity. The forced expression of *cmpABCD* did not significantly increase the CO_2 uptake capabilities of the cells. These findings demonstrated that the *cmpABCD* genes encode a high-affinity HCO_3^- transporter. A deletion mutant of *cmpAB* (M42) retained low CO_2-inducible activity of HCO_3^- transport, indicating the occurrence of HCO_3^- transporter(s) distinct from the one encoded by *cmpABCD*. HCO_3^- uptake by low-CO_2-induced M42 cells showed lower affinity for external HCO_3^- than for wild-type cells under the same conditions, showing that the HCO_3^- transporter encoded by *cmpABCD* has the highest affinity for HCO_3^- among the HCO_3^- transporters present in the cyanobacterium. This appears to be the first unambiguous identification and description of a primary active HCO_3^- transporter.

Cyanobacteria possess a CO_2-concentrating mechanism (CCM), which elevates the CO_2 concentration around the active site of ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) and thereby enables efficient CO_2 fixation despite the low affinity and selectivity of their Rubisco for CO_2 (1, 2). The CCM involves the abilities to actively transport inorganic C (CO_2 and HCO_3^-; designated C_i) into the cell, to accumulate C_i as HCO_3^- in the cytoplasm, and to effectively convert HCO_3^- into CO_2 in carboxysomes, the polyhedral inclusion bodies to which Rubisco is localized. The C_i-transporting mechanism plays a major role in adaptation of cyanobacteria to changing availability of C_i. Cells grown under high-CO_2 conditions (1–5% CO_2, vol/vol) have low-affinity C_i transport activities and incubation of the cells under low-CO_2 conditions (0.035% CO_2 or less, vol/vol) induces expression of high-affinity C_i transport activities. Physiological studies have suggested the occurrence of multiple forms of C_i transporters, which are distinct in substrate specificity (HCO_3^- or CO_2), affinity for the substrate, inducibility by C_i limitation, and requirement for Na^+ (1, 2). The genes for C_i transporters have been sought after for some time, partly because of their potential for improving the nitrogen and water-use efficiency of photosynthesis when introduced into higher plants performing C3-type photosynthesis.

Genetic analysis of cyanobacterial mutants that require high-CO_2 concentrations for growth has identified >10 genes directly related to the CCM, but most of them are involved in assembly and functioning of the carboxysome (2). The *Synechocystis* sp. strain PCC 6803 mutants with impaired C_i transport activities are defective either in the *ndh* genes [encoding the subunits of NAD(P)H dehydrogenase] that are presumably involved in energization of C_i transporters (3), or in the *pxcA* (*cotA*) gene, which is required for H^+ extrusion into the external medium and is indirectly involved in CO_2 transport (4). Analysis of high-CO_2-requiring mutants of *Synechococcus* sp. strain PCC 7942, on the other hand, identified a gene (*ictB*) necessary for HCO_3^- transport (5). Targeted inactivation of this gene results in loss of HCO_3^--transporting activity in cells grown under high-CO_2 conditions and practically abolishes induction of high-affinity HCO_3^- transport activity under low-CO_2 conditions (5). However, *ictB* is unlikely to encode a HCO_3^- transporter, because the profound effect of the *ictB* mutation on HCO_3^--transporting activity is incompatible with the presumed occurrence of multiple HCO_3^- transporters. Thus, no mutants defective in C_i transporters have been identified to date.

Studies on the membrane proteins of *Synechococcus* sp. strain PCC 7942, on the other hand, identified a 42-kDa plasma membrane protein synthesized under C-limited conditions (6). The coinduction of the protein, concomitantly with enhanced C_i-transport activity, suggested that the protein may play a role in C_i transport (6, 7), but at the time, this possibility was considered unlikely because the protein deduced from the nucleotide sequence of the gene (*cmpA*) was largely hydrophilic and a deletion mutant (M42) of the gene showed low-CO_2-inducible activities of CO_2 and HCO_3^- transport (8). Later studies showed that *cmpA* forms a gene cluster with three genes located downstream (*cmpB*, *cmpC*, and *cmpD*) that encode a set of proteins comprising a membrane complex of an ATP-binding cassette (ABC) transporter (ref. 9, GenBank accession no. D26358). The genes *nrtA*, *nrtB*, *nrtC*, and *nrtD*, which are strongly similar to *cmpA*, *cmpB*, *cmpC*, and *cmpD*, respectively, were shown to encode a nitrate/nitrite bispecific transporter (10, 11), with the *nrtA* gene product acting as the membrane-anchored substrate-binding protein (12). Thus, the 42-kDa protein is likely to be the substrate-binding protein of a transporter encoded by *cmpABCD*. The presumed occurrence of multiple C_i transporters would account for the presence of inducible C_i transport activities in M42. On the basis of these considerations, we have reinvestigated the role of the *cmp* genes. The genes are shown to constitute a low-CO_2-inducible operon. By selective induction of the *cmp* genes under high-CO_2 conditions, by using a nitrogen-regulated promoter of the *nirA* operon (13), it is shown that *cmpABCD* encodes a high-affinity HCO_3^- transport system.

Abbreviations: ABC, ATP-binding cassette; CCM, CO_2-concentrating mechanism; Chl, chlorophyll; C_i, inorganic C; WT, wild type.

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Materials and Methods

Strains and Growth Conditions. Cells of the wild-type (WT) *Synechococcus* sp. strain PCC 7942, mutant M42 (8), and a genetically engineered mutant derived herein (see below) were grown photoautotrophically at 30°C under continuous illumination provided by fluorescent lamps. The basal medium used was a nitrogen-free medium obtained by modification of BG11 medium (14) as described previously (15). Nitrate-containing medium and ammonium-containing medium were prepared by addition of 15 mM KNO_3 or 3.75 mM $(NH_4)_2SO_4$, respectively, to the basal medium. The media were buffered with either 20 mM Hepes-KOH (pH 8.0) or 10 mM 1,3-bis[tris(hydroxymethyl)methylamino]propane (BTP)-HCl (pH 8.0). When appropriate, kanamycin and spectinomycin were added to the media at 15 and 10 μg/ml, respectively.

The cultures were routinely maintained under high-CO_2 conditions, i.e., aeration with 2% (vol/vol) CO_2 in air, under illumination at 90 μmol of photons $m^{-2}\cdot s^{-1}$. For the experiments involving induction of C_i transport activities in the CMP+ mutant under high-CO_2 conditions, cultures were grown at a light intensity of 250 μmol of photons $m^{-2}\cdot s^{-1}$. For the other experiments, involving induction of C_i transport activities by C_i limitation, cells were grown under a light intensity of 90 μmol of photons $m^{-2}\cdot s^{-1}$ so as to minimize photoinhibitory damage to the cells, which becomes prominent at high light intensities and low CO_2 concentrations (16, 17). For transfer of ammonium-grown cells to nitrate-containing medium under the high-CO_2 conditions, cells grown to the mid-logarithmic phase of growth were collected by centrifugation at 5,000 $\times$ *g* for 5 min at 25°C, washed twice with the nitrogen-free medium by resuspension and recentrifugation, inoculated into nitrate-containing medium, and incubated under the same general conditions as before. For transfer of high-CO_2-grown cells to low-CO_2 conditions, cells were grown in nitrate-containing medium, collected by centrifugation as described above, washed twice with the growth medium by resuspension and recentrifugation, inoculated into fresh nitrate-containing medium, and aerated with air containing 0.002–0.005% (vol/vol) CO_2 under the preexisting conditions.

Nitrogen-Regulated Expression of *cmpABCD* in *Synechococcus* Cells Under High-CO_2 Conditions. To promote nitrogen-regulated expression of the *cmpABCD* genes under high-CO_2 conditions, a mutant CMP+ (Fig. 1) was constructed by replacing the promoter of the *cmp* operon with that of the *nirA* operon as follows. A 0.59-kbp DNA fragment, carrying nucleotides +1 to +579 of the *cmpA*-coding region, was amplified by PCR and cloned into pT7Blue T-Vector (Novagen). Two bases of the sense primer used, corresponding to nucleotides −2 and −1 with respect to *cmpA* initiation codon, had been changed from G and T in the original *cmpA* sequence to T and C, respectively, to create a *Bsp*HI recognition site at the translation start site. After confirmation of nucleotide sequence, the *cmpA* fragment was excised from the plasmid with *Bsp*HI and *Eco*RI and assembled in pUC19 with a 0.56-kbp *Hin*dIII/*Nco*I fragment of the *nirA* upstream region. In the resulting plasmid, the *nirA* upstream region (nucleotides −560 to −1 with respect to the *nirA* initiation codon) was fused with the *cmpA*-coding region (nucleotides +1 to +579 with respect to the *cmpA* initiation codon). A spectinomycin/streptomycin resistance (*spe*^{r}) gene cassette excised from plasmid pRL463 (18) was subsequently ligated between the two *Bal*I sites in the *nirA* upstream region to replace the 46-bp region extending from nucleotide −368 to −323 with respect to the initiation codon. A 1.6-kbp *Eco*47III fragment excised from the resulting plasmid, carrying, sequentially, nucleotides −410 to −369 of the *nirA* upstream region, the *spe*^{r} gene cassette, nucleotides −322 to −1 of *nirA* upstream region, and nucleotides +1 to +66 of *cmpA*-coding region, was ligated between nucleotides −143 and +67 of *cmpA* on a 1.7-kbp *Synechococcus* DNA fragment that had been cloned separately in pUC19. The resulting plasmid was used to transform the WT strain to spectinomycin resistance through homologous recombination. The transformants were allowed to grow on solid medium supplemented with 15 μg of spectinomycin per ml. After three serial streak-purifications to segregate homozygous mutants (19), genomic DNA was isolated from the selected clones and analyzed by Southern hybridization, using the *spe*^{r} gene cassette as a probe, and PCR to confirm the insertion of the *spe*^{r} gene cassette and the *nirA* promoter in the *cmpA* regulatory region, respectively.

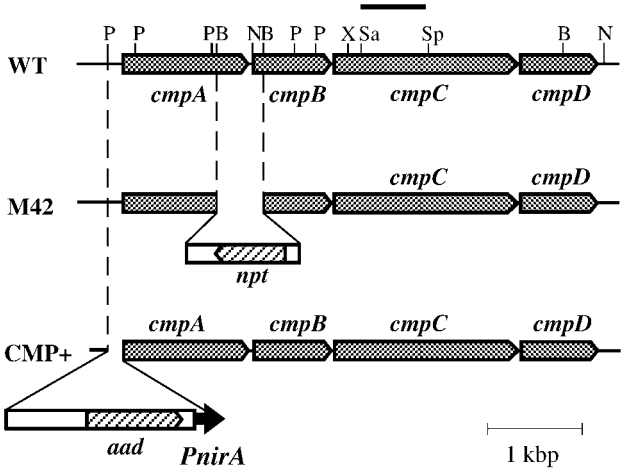

Fig. 1. Comparison of the structures of the *cmp*-genomic region in WT and in the M42 (Δ*cmpAB*::*kan*^{r}) and CMP+ (*PnirA*::*cmpABCD*) mutants of *Synechococcus* sp. strain PCC7942. The bar above the map shows the probe region used for Northern hybridization analysis. The open bars represent the antibiotic-resistance gene cassettes and the hatched bars show the location and orientation of the kanamycin-resistance gene (*npt*) and the *spe*^{r} gene (*aad*). The restriction endonuclease sites are abbreviated as follows: B, *Bgl*II; N, *Nco*I; P, *Pst*I; Sa, *Sal*I; Sp, *Sph*I; and X, *Xba*I.

Measurements of the Initial Rate of HCO_3^- Uptake After Onset of Light. Cells were collected by centrifugation as described above, washed twice by recentrifugation and resuspension in the assay buffer (50 mM BTP-HCl, pH 9.0/15 mM NaCl/0.3 mM $MgSO_4$/0.26 mM $CaCl_2$/0.22 mM K_2HPO_4), which had been sparged with a mixture of N_2 and O_2 (4:1, vol/vol) for >4 h, and finally suspended in the assay buffer at a chlorophyll (Chl) concentration of 2.9 μg/ml. After incubation under illumination at 30°C for 30 min in a tightly sealed tube, aliquots of 0.39 ml of the cell suspension were transferred to microcentrifuge tubes. $NaH^{14}CO_3$ was added in the dark to the cell suspensions to give a final HCO_3^- concentration of 100 μM. Immediately after the addition of HCO_3^-, 0.25 ml of the cell suspension was sucked up into a transparent micropipette tip, and HCO_3^- uptake was started by onset of illumination at 400 μmol of photons $m^{-2}\cdot s^{-1}$ provided through optical fibers. The uptake reaction was terminated by rapid filtration of the cells onto a glass filter (GF/B, Whatman) by suction, followed by immediate washing of the filter with 5 ml of the assay buffer, and the radioactivity retained on the filter was measured with a scintillation counter.

Measurements of the Rates of CO_2 and HCO_3^- Uptake During Steady-State Photosynthesis. Cells were harvested, washed, and suspended in the assay buffer as described above, except that the pH of the assay buffer was 8.2. The steady-state rates of gross CO_2 and net HCO_3^- uptake were measured by a mass spectrometric

disequilibruim technique in an aqueous phase-sampling mass spectrometer as described previously (20) under illumination at 300 μmol of photons $m^{-2}\cdot s^{-1}$.

Isolation and Analysis of DNA and RNA. Chromosomal DNA was extracted and purified from the *Synechococcus* cells as described by Williams (19). Manipulations and analyses of DNA were performed according to standard protocols (21). Total RNA was extracted and purified from *Synechococcus* cells by the method of Aiba *et al.* (22). For Northern hybridization analysis, a 0.7-kbp *Sal*I/*Sph*I fragment of *cmpC* was used as a probe (Fig. 1).

Immunoblotting Analysis. Cytoplasmic (plasma) membrane was purified from *Synechococcus* cells as described (23). Membrane samples amounting to 5 μg of protein were solubilized in the sample buffer for SDS/PAGE (24) at room temperature for 30 min. After gel electrophoresis in the buffer system of Laemmli (24), polypeptides were electrotransferred to a poly(vinylidene difluoride) membrane and allowed to react with IgG against CmpA (8). A goat anti-rabbit IgG-alkaline phosphatase conjugate (Bio-Rad) was used as the second antibody and detected by the color development reaction catalyzed by alkaline phosphatase with nitroblue tetrazolium and 5-bromo-4-chloro-3-indolyl phosphate as substrates.

Other Methods. Chl and protein were determined according to Mackinney (25) and Lowry *et al.* (26), respectively.

Results

Expression of *cmp* Genes in WT and the CMP+ Mutant. Northern hybridization analysis using a *cmpC*-specific probe showed that expression of the gene is induced by C_i limitation in the WT cells of *Synechococcus* sp. PCC 7942 (Fig. 2*A*, lanes 1 and 2). The hybridization profile showed a discrete 5.2-kb band preceded by a smaller smeary signal, indicating that the gene is transcribed as a 5.2-kb mRNA, which is rapidly degraded. The 5.2-kb mRNA is close to the calculated size of the *cmpABCD* gene cluster, 5.1 kb, and probes specific to other *cmp* genes yielded essentially the same hybridization profiles (not shown), verifying that the *cmpABCD* genes constitute a low-CO_2-inducible operon in the WT strain. Although WT cells did not express the *cmpABCD* operon under high-CO_2 conditions irrespective of the nitrogen conditions tested (Fig. 2*A*, lane 1 and Fig. 2*B*, lanes 1 and 2), the CMP+ cells accumulated a large amount of *cmpABCD* transcript under high-CO_2 conditions when transferred from ammonium-containing medium to nitrate-containing medium (Fig. 2*B*, lane 4). The cells accumulated insignificant amounts of *cmpABCD* transcript when grown with ammonium (Fig. 2*B*, lane 3). These results verified that transcription of *cmpABCD* in CMP+ is under the control of the *nirA* operon promoter (13, 27). Immunoblot analysis of the cytoplasmic membrane proteins showed that nitrate-grown CMP+ cells accumulated the 42-kDa protein under high CO_2 to an amount comparable with that in the low-CO_2-induced WT cells (Fig. 2*C*, lanes 2 and 4), confirming effective translation and processing from the *cmpABCD* transcript under high-CO_2 conditions. Although minute levels of *cmpABCD* transcript (Fig. 2*B*, lane 3) were detected, ammonium-grown CMP+ cells accumulated small amounts of the 42-kDa protein (Fig. 2*C*, lane 3), confirming that the *nirA* operon promoter is not completely suppressed.

HCO_3^- Uptake Activity of the CMP+ Mutant. Fig. 3 shows the time course for HCO_3^- uptake by WT and CMP+ cells after onset of illumination at pH 9 and 100 μM external HCO_3^- concentration, under which conditions HCO_3^-, comprising $\approx$99.8% of C_i in medium, is for practical purposes considered as the C_i species actively transported into the cell. Whereas the WT cells grown under high CO_2 on nitrate took up negligible amounts of HCO_3^- during the first 20 s after illumination (<20 nmol per mg of Chl), the CMP+ cells grown under the same conditions accumulated 260 nmol of HCO_3^- per mg of Chl. The rate of HCO_3^- uptake by high-CO_2/nitrate-grown CMP+ cells was calculated to be 47 μmol per mg of Chl per h, which corresponded to one-half of that in the low CO_2/nitrate-grown WT cells, namely 98 μmol per mg

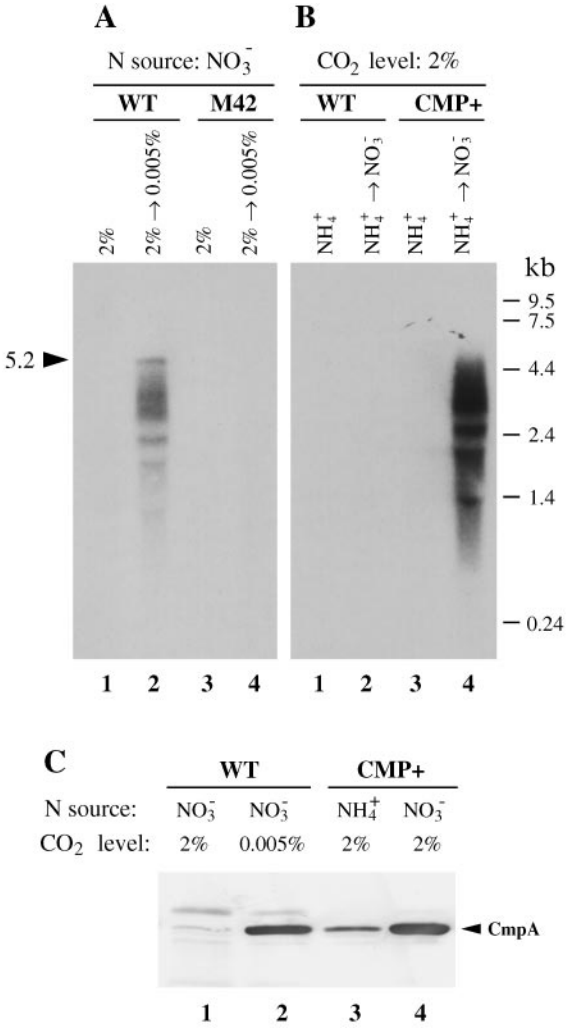

Fig. 2. (*A*) Northern hybridization analysis of total RNA from *Synechococcus*, showing the effects of CO_2 conditions on expression of the *cmp* operon. *Synechococcus* cells were grown with NO_3^- under high-CO_2 conditions (2% CO_2 in air) and transferred to low-CO_2 conditions (0.005% CO_2 in air). RNA samples (10 μg per lane) from WT (lanes 1 and 2) and the M42 mutant (lanes 3 and 4), extracted before (lanes 1 and 3) and 30 min after (lanes 2 and 4) the transfer, were denatured with formamide, separated on a 1.2% agarose-formaldehyde gel, transferred to a positive-charged nylon membrane (Hybond N+, Amersham), and hybridized with a ^{32}P-labeled *cmpC*-specific probe. (*B*) Northern hybridization analysis of total RNA, showing the nitrogen-regulated expression of the *cmp* gene cluster under high CO_2 in the CMP+ mutant. *Synechococcus* cells were grown with NH_4^+ and transferred to NO_3^--containing medium under high-CO_2 conditions. RNA samples (10 μg per lane) from WT (lanes 1 and 2) and CMP+ (lanes 3 and 4), extracted before (lanes 1 and 3) and 30 min after (lanes 2 and 4) the transfer, were analyzed as in *A*. (*C*) Immunoblotting analysis of CmpA in the plasma membrane of *Synechococcus* grown under constant C and nitrogen conditions. Plasma membrane samples from WT cells grown under high CO_2 (lane 1) and low CO_2 (lane 2) conditions in NO_3^--containing medium and those from the CMP+ cells grown under high-CO_2 conditions in NH_4^+- (lane 3) and NO_3^-- (lane 4) containing media were compared. Membrane proteins (5 μg per sample) were solubilized with SDS, fractionated by SDS/PAGE (10% gel), and electrotransferred to poly(vinylidene difluoride) membrane for immunostaining.

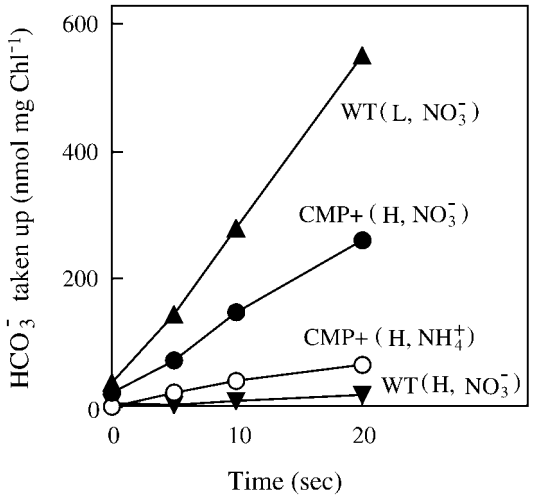

Fig. 3. Uptake of HCO_3^- by high-CO_2-grown cells (H) of WT (▼) and the CMP+ mutant (○, ●) and low-CO_2-grown cells (▲) of WT (H) in the light. Cells were grown with NH_4^+ (○) or NO_3^- (●, ▼, ▲) as the nitrogen source. Uptake was initiated by illumination immediately after the addition of 100 μM $H^{14}CO_3^-$ to the cell suspensions. The amount of HCO_3^- taken up by the cells was determined from the total amount of ^{14}C accumulated in the cell. Assays were done at 30°C and pH 9.0.

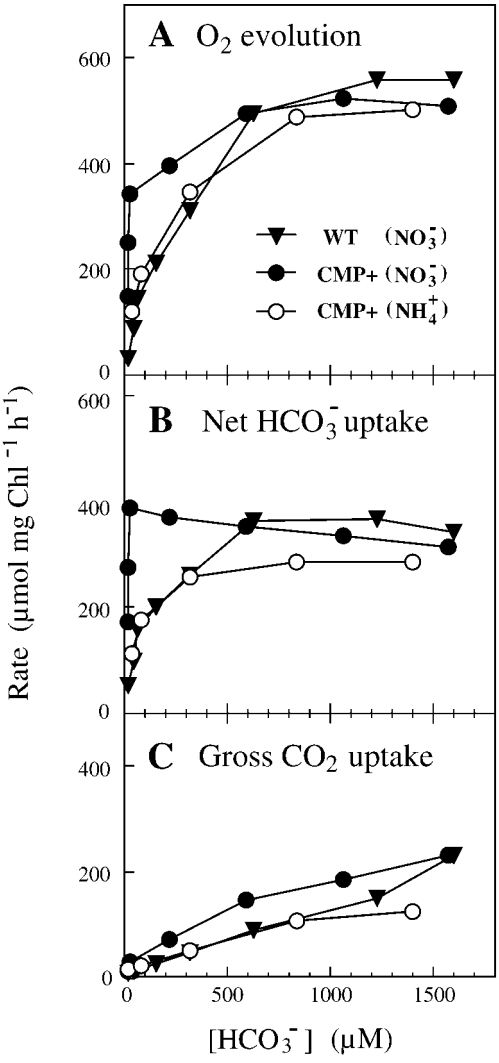

Fig. 4. The rate of O_2 evolution (*A*), net HCO_3^- uptake (*B*), and gross CO_2 uptake (*C*) as a function of the HCO_3^- concentration in medium during steady-state photosynthesis in WT (▼) and the CMP+ mutant (○ and ●). Cells were grown under high-CO_2 conditions at a light intensity of 250 μmol of photons $m^{-2}{\cdot}s^{-1}$ with NH_4^+ (○) or NO_3^- (● and ▼) as the nitrogen source. Assays were done at 30°C and pH 8.2.

of Chl per h. By contrast, when grown with ammonium, the HCO_3^- uptake rate of the CMP+ cells was only 20% of that in the nitrate-grown cells and only 10% of the rate in WT cells adapted to low CO_2. These results demonstrate that nitrogen-regulated expression of the *cmp* operon correlates with an elevated capacity to transport low concentrations of HCO_3^- in the CMP+ mutant under high-CO_2 conditions.

Fig. 4*A* shows the dependence of the rates of O_2 evolution on external HCO_3^- during steady-state photosynthesis of high-CO_2-grown cells of CMP+ and WT. In WT cells and ammonium-grown CMP+ cells, the O_2 evolution rates showed a saturation-type kinetics with respect to the external HCO_3^- concentration, with the $K_{1/2}$ value (the concentration of HCO_3^- required for the one-half maximal response) being 260 μM and 170 μM, respectively. The O_2 evolution rate of nitrate-grown CMP+, on the other hand, showed a biphasic response to external HCO_3^-; the rate sharply increased with increasing external HCO_3^- up to 30 μM concentration and then gradually increased to reach a maximum level similar to that in WT and ammonium-grown CMP+ cells at ≈1 mM external HCO_3^- concentration. The $K_{1/2}$ value for the first phase was 16 μM. These findings show that nitrate-grown CMP+ cells have an efficient mechanism to use low concentrations of external C_i for photosynthesis. When the steady-state rates of net HCO_3^- and gross CO_2 uptake were plotted as functions of external HCO_3^- (Fig. 4 *B* and *C*), it was clear that the nitrate- and ammonium-grown CMP+ cells differed greatly in their ability to take up low concentrations of HCO_3^- into the cell. The $K_{1/2}$ value for HCO_3^- uptake was 15 μM and 60 μM in the nitrate- and ammonium-grown cells, respectively. These findings demonstrated that induction of *cmpABCD* genes under high-CO_2 conditions led to selective expression of a high-affinity, HCO_3^- transport activity. The $K_{1/2}$ value for HCO_3^- uptake in ammonium-grown CMP+ was smaller than that in WT cells, 120 μM, presumably due to the low-level expression of the *cmpABCD* genes in the presence of ammonium (Fig. 2*C*, lane 3). We therefore conclude that the *cmpABCD* gene cluster encodes an ABC-type HCO_3^- transporter, designated BCT1.

HCO_3^- Uptake Activity of the *cmp* Deletion Mutant. Fig. 5 shows the rates of O_2 evolution (*A*), net HCO_3^- uptake (*B*), and gross CO_2 uptake (*C*) of the high-CO_2-grown and low-CO_2-adapted cells of WT and M42 during steady-state photosynthesis. The high-CO_2-grown cells of WT and M42 were essentially the same in their activity to take up CO_2 and HCO_3^- and to photosynthesize over a range of HCO_3^- concentrations. Incubation of the cells under C_i-limited conditions increased the HCO_3^- uptake activity in both WT and M42 but in different ways. The $K_{1/2}$ value for HCO_3^- uptake in WT cells decreased from 300 μM to 11 μM, similar to previously reported data (28); however, in the M42 mutant, defective in the *cmpABCD* genes, the $K_{1/2}$ value declined to only 33 μM. Also, the maximal rate of HCO_3^- uptake was increased by 50% in WT after incubation under low CO_2, but there was no change in the maximal rate of HCO_3^- uptake in M42 (Fig. 5*B*). The capacity for CO_2 transport was marginally larger in M42 than in WT after growth under the C_i-limited conditions (Fig. 5*C*). The maximum rate of O_2 evolution was similar in low CO_2-grown WT and M42 cells, but M42 showed a lower affinity for HCO_3^- ($K_{1/2}$ = 70 μM) than WT cells ($K_{1/2}$ = 15 μM). These results are consistent with the loss of BCT1 activity in M42 cells, and at the same time, confirm the existence of a second, low-CO_2-inducible, HCO_3^- transporter in the

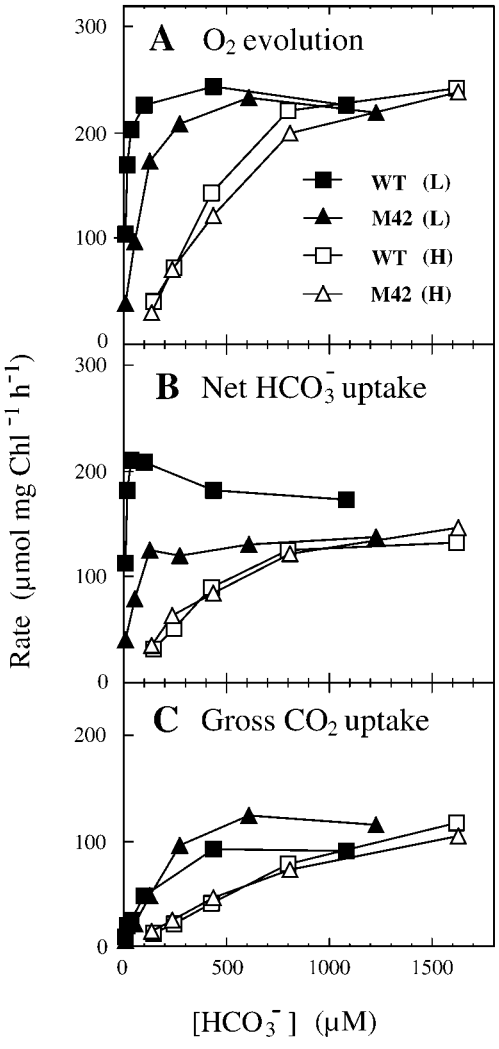

Fig. 5. The rate of O_2 evolution (*A*), net HCO_3^- uptake (*B*), and gross CO_2 uptake (*C*) as a function of the HCO_3^- concentration in medium during steady-state photosynthesis in WT (□ and ■) and the M42 mutant (△ and ▲). Cells were grown under high-CO_2 (□ and △; H) and low-CO_2 (■ and ▲; L) conditions at a light intensity of 90 μmol of photons $m^{-2}{\cdot}s^{-1}$. Assays were done as described in Fig. 4.

BCT1-deficient mutant (29). The larger $K_{1/2}$ value for HCO_3^- uptake in low-CO_2-induced M42 cells indicates that BCT1 has the highest affinity for HCO_3^- of the HCO_3^- transport activities present in *Synechococcus* sp. PCC 7942.

Discussion

Studies on the cyanobacterial CCM have so far depended on loss-of-function analysis of the properties of mutants defective in specific genes. The approach, however, has limitations in studies of biochemical functions encoded by functionally redundant genes, such as the multiple transporters used in the uptake of C_i. We, therefore, performed a gain-of-function analysis to ascertain the role of *cmpABCD*. A nitrogen-regulated promoter allowed expression of the genes under high-CO_2 conditions (Fig. 2), in which high-affinity C_i transport activities are normally not induced. Measurements of the initial rate of HCO_3^- uptake after onset of light and of the steady-state rate of HCO_3^- uptake in the light showed that the selective induction of *cmpABCD* resulted in expression of high-affinity HCO_3^- transport activity (Figs. 3 and 4), demonstrating that the gene cluster encodes a high-affinity HCO_3^- transporter that we have now named BCT1. BCT1 is the first ABC transporter known to transport HCO_3^- and appears to be the first primary-active HCO_3^- transporter, although Na^+/HCO_3^- cotransporters and HCO_3^-/anion exchangers have been characterized in mammals.

We previously thought that *cmpA* was not involved in HCO_3^- transport on the basis of the presence of inducible C_i transport activities in M42 (8). The present results, obtained from mass spectrometric analysis of the steady-state rates of C_i uptake, confirmed the existence of the inducible CO_2 and HCO_3^--transporting activities in M42, but in accordance with the loss of BCT1, the mutant was impaired specifically in induction of a high-affinity HCO_3^- uptake mechanism (Fig. 5). These findings indicate that *Synechococcus* sp. strain PCC7942 has at least two low CO_2-inducible HCO_3^- transporters, with BCT1 having the highest affinity for HCO_3^-. We predict that BCT1 will be of considerable ecological significance in cyanobacteria. The remaining HCO_3^- transport activity in M42 is presumably predominated by a HCO_3^- transporter(s) capable of a fast induction response (within 10 min) that is initially independent of transcription–translation events (30).

Recently, a gene (*ictB*) essential for HCO_3^- transport has been cloned from a high CO_2-requiring mutant of *Synechococcus* sp. strain PCC 7942 and presumed to encode a HCO_3^- transporter on the basis of the hydrophobic nature of the deduced protein (5). However, inactivation of *ictB* practically abolishes induction of high-affinity HCO_3^- transport activity under low-CO_2 conditions (5), meaning that all the low-CO_2-inducible HCO_3^--transporting mechanisms, encoded by *cmpABCD* and the other(s) remaining in M42, are missing or nonfunctional in the *ictB* mutant. Therefore, *ictB* appears to be epistatic to *cmpABCD* and is unlikely to encode a HCO_3^- transporter by itself. The essential role of *ictB* in HCO_3^- transport suggests that the product of *ictB* may be involved in other processes, such as the transport of other ion(s) for compensating the large flux of negative electric charge across the plasma membrane during the uptake of HCO_3^-. Further work is required for elucidation of the biochemical function of the product of the *ictB* gene.

In the nitrate/nitrite transporter of *Synechococcus* sp. strain PCC 7942, the NrtA protein has been shown to be the substrate-(nitrate and nitrite) binding lipoprotein anchored to the cytoplasmic membrane (12). CmpA is 46.5% identical to NrtA (31) and has a putative signal peptide typical of a lipoprotein (12), suggesting that the protein is a membrane-anchored lipoprotein and functions as the HCO_3^--binding protein. On the other hand, one of the ATP-binding subunits of the nitrate/nitrite transporter, NrtC, has a distinct C-terminal domain required for ammonium-promoted inhibition of nitrate/nitrite transport (32). Because CmpC also has a C-terminal domain, which is 30% identical to the corresponding domain of NrtC (9), it is inferred by analogy that CmpC has a regulatory role in HCO_3^- transport. Currently it is unknown what kind of regulation the BCT1 transporter is subject to. Biochemical and molecular biological studies on the CmpA and CmpC proteins are being performed to elucidate the structure–function relationships of the HCO_3^- transporter.

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