Identification of a SulP-type bicarbonate transporter in marine cyanobacteria

G. Dean Price*†, Fiona J. Woodger*, Murray R. Badger*, Susan M. Howitt‡, and Loraine Tucker*

*Molecular Plant Physiology Group, Research School of Biological Sciences, and ‡School of Biochemistry and Molecular Biology, Science Faculty, Australian National University, P.O. Box 475, Canberra ACT 0200, Australia

Edited by Sallie W. Chisholm, Massachusetts Institute of Technology, Cambridge, MA, and approved October 31, 2004 (received for review July 19, 2004)

Cyanobacteria possess a highly effective CO_2-concentrating mechanism that elevates CO_2 concentrations around the primary carboxylase, Rubisco (ribulose-1,5-bisphosphate carboxylase/oxygenase). This CO_2-concentrating mechanism incorporates light-dependent, active uptake systems for CO_2 and HCO_3^-. Through mutant studies in a coastal marine cyanobacterium, *Synechococcus* sp. strain PCC7002, we identified *bicA* as a gene that encodes a class of HCO_3^- transporter with relatively low transport affinity, but high flux rate. BicA is widely represented in genomes of oceanic cyanobacteria and belongs to a large family of eukaryotic and prokaryotic transporters presently annotated as sulfate transporters or permeases in many bacteria (SulP family). Further gain-of-function experiments in the freshwater cyanobacterium *Synechococcus* PCC7942 revealed that *bicA* expression alone is sufficient to confer a Na^+-dependent, HCO_3^- uptake activity. We identified and characterized three cyanobacterial BicA transporters in this manner, including one from the ecologically important oceanic strain, *Synechococcus* WH8102. This study presents functional data concerning prokaryotic members of the SulP transporter family and represents a previously uncharacterized transport function for the family. The discovery of BicA has significant implications for understanding the important contribution of oceanic strains of cyanobacteria to global CO_2 sequestration processes.

SulP transporters | CO_2 sequestration | photosynthesis

It is estimated that some 50% of global primary productivity occurs in the oceans, and marine cyanobacteria contribute significantly to this global CO_2 sequestration process (1). For example, in open oceans located between 40°N and 40°S, photosynthetic CO_2 fixation is dominated by marine cyanobacteria of the *Synechococcus* and *Prochlorococcus* genera, and together these species perform 30–80% of primary production (2, 3). The largest nutrient uptake flux encountered by marine cyanobacteria is for dissolved inorganic carbon (Ci), yet relatively little is known about the process of Ci accumulation for photosynthesis in the oceanic cyanobacteria, in contrast to our knowledge of freshwater strains.

In aquatic systems, Ci exists mainly as two slowly interconvertible forms, CO_2 and HCO_3^-. In response to unique restrictions on the rate of CO_2 supply in the aquatic environment, cyanobacteria have evolved a very efficient mechanism for capturing CO_2 and HCO_3^- for photosynthetic fixation into sugars. The Ci-capturing mechanism functions as a CO_2-concentrating mechanism because it effectively concentrates CO_2 around the main CO_2-fixing enzyme, ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco). In the best characterized species, mostly freshwater cyanobacterial strains, the CO_2-concentrating mechanism consists of several active uptake systems for CO_2 and HCO_3^-, plus a unique microcompartment called the carboxysome that contains the CO_2-fixing enzyme, Rubisco (4–6).

In recent years, the availability of a number of complete genomic databases for a range of cyanobacteria has made it possible to identify likely homologs of known Ci transporters in marine species of cyanobacteria (7, 8). An interesting observation is that marine cyanobacteria do not seem to possess the high affinity HCO_3^- transporter (encoded by *cmpABCD*, a traffic ATPase) that is present in many freshwater species (7). Potential homologs of the Na^+-dependent, SbtA-type HCO_3^- transporter have been noted as present in some oceanic strains (7, 8), but they share low sequence homology with SbtA proteins from *Synechocystis* PCC6803 (freshwater) and, as yet, have not been proven to transport HCO_3^-.

In this study, we have identified a previously undiscovered class of HCO_3^- transporter, here named BicA, that is well represented in the genomes of oceanic cyanobacteria. BicA belongs to a large family of eukaryotic and prokaryotic anion transporters (9) where many of the eubacterial members are presently annotated as putative sulfate transporters (TC 2.A.53). Here, we report a previously uncharacterized function for a prokaryotic member of the SulP transporter family.

Materials and Methods

Cyanobacterial Strains and Culture Conditions. *Synechococcus* PCC7942 was cultured as described (10). *Synechococcus* PCC7002 was cultured in BG-11 medium supplemented with 16 μg·liter^{-1} biotin, 20 mM $MgSO_4$, 8 mM KCl, and 300 mM NaCl, at 28°C with a light intensity of 120 μmol photons·m^{-2}·s^{-1}. Aeration of both strains was delivered through pipettes (1-mm annulus) at a flow rate of 0.15 liters·min^{-1}.

Gene Inactivation in *Synechococcus* PCC7002. Genes for *sbtA*, *sul2*, and *bicA* (*sul3*), with flanking regions of 0.5–1.0 kb, were PCR amplified from *Synechococcus* PCC7002 genomic DNA and ligated into pGEM-T (Promega). Sequence data were obtained from the draft genome sequence for *Synechococcus* PCC7002 (GenBank genome NC_003488) compiled by D. A. Bryant and J. Zhou (Fig. 7, which is published as supporting information on the PNAS web site, shows the BicA protein sequence); primer sequences are included in Table 4, which is published as supporting information on the PNAS web site. The drug resistance markers kanamycin [Kan (11)], chloramphenicol [Cm (12)], and spectinomycin [Sp (13)] were cloned in the *Hin*cII site of *sbtA*, *Bal*I-*Hpa*I deletion in *sul2*, and *Eco*RI-*Bal*I deletion of *bicA* (*sul3*), respectively, in parallel to the direction of transcription. Transformation of *Synechococcus* PCC7002 cells was as described (11). Levels of Kan, Cm, and Sp were 200, 8, and 10 μg·ml^{-1} in solid media and 150, 7, and 5 μg·ml^{-1} in liquid media.

Expression Constructs for *Synechococcus* PCC7942. Coding regions for *bicA* (*sul3*) and *sul2* from *Synechococcus* PCC7002 (*bicA*-7002 and *sul2*-7002), *Synechococcus* WH8102 (*bicA*-WH and *sul2*-WH), and *Synechocystis* PCC6803 (*bicA*-6803 only) were PCR amplified from genomic DNA, ligated into pGEM-T, verified by DNA sequencing, and then cloned into the shuttle

This paper was submitted directly (Track II) to the PNAS office.

Abbreviations: Ci, inorganic carbon; $K_{0.5}$, concentration required for half maximal response.

†To whom correspondence should be addressed. E-mail: dean.price@anu.edu.au.

vector pSE4 (14). Sequence coordinates for *Synechococcus* PCC7002 clones are presented in Table 5, which is published as supporting information on the PNAS web site. Sequences for *Synechococcus* WH8102 and *Synechocystis* PCC6803 were obtained from Cyanobase (www.kazusa.or.jp); *bicA*-WH, *sul2*-WH, and *bicA*-6803 have gene identifiers of *sll0834*, *slr0096*, and *SynW1524*. Primer sequences and key cloning sites are shown in Table 6, which is published as supporting information on the PNAS web site. *Synechococcus* PCC7942 was transformed as described (15) with Sp selection at 10 and 7 $\mu g \cdot ml^{-1}$ on solid and liquid media, respectively. Cells usually were grown in the presence of 10 mM NH_4^+ to limit overexpression.

Physiological Measurements. Prepared cells were analyzed by mass spectrometry as described (10, 16). Assays for net O_2 evolution were performed at 30°C at a chlorophyll density of 2 $\mu g \cdot ml^{-1}$ in the appropriate version of BG11 medium buffered with 50 mM BisTrisPropane-HCl (pH 8, 9, or 9.3), with 17 mM $NaNO_3$ replaced with 20 mM NaCl. Changes for both O_2 and CO_2 were monitored at a light intensity of 600 μmol photons$\cdot m^{-2} \cdot s^{-1}$. Active species uptake of $H^{14}CO_3^-$ was carried out at pH 9 (25°C) with 15-s uptake periods (17) terminated by silicone oil centrifugation-filtration (18). $KH^{14}CO_3$ aliquots were added from a 25 mM stock (pH 9.5). The rate of CO_2 supply from HCO_3^- at pH 9 was calculated by applying an experimentally determined rate constant (19) of 0.603 min^{-1}.

Real-Time RT-PCR Assays. All *Synechococcus* PCC7002 sequences were obtained from the draft genome sequence by comparisons with known genes from *Synechococcus* PCC7942 or *Synechocystis* PCC6803. Real-time RT-PCR assays using gene-specific primer pairs (Table 5), and with SYBR Green I to monitor product formation, were performed as described by Woodger *et al*. (10) except that a Rotorgene 3000 (Corbett Research, Sydney, Australia) was used and the accompanying software was used to analyze the second derivative of the raw fluorescence data collected for each sample during a run. Fold changes in transcript abundance relative to a basal condition were calculated after the method of Liu and Saint (20) by using two normalizing genes, *rnpA* (RNase P) and *petB* (Cyt b_6).

Results

Identification of *bicA* as a Gene Encoding a Second HCO_3^- Transporter in *Synechococcus* PCC7002. Initial analysis of an *sbtA* mutant in *Synechococcus* PCC7002 grown under Ci-limitation showed a response to added Ci that was intermediate between the responses of WT cells grown under Ci-limitation and Ci-excess (Fig. 1), indicating the presence of another HCO_3^- transporter of moderate transport affinity. Marine cyanobacteria so far sequenced (including *Synechococcus* PCC7002) do not possess a *cmpABCD*-type HCO_3^- transporter (7). A draft genome sequence for *Synechococcus* PCC7002 has been constructed by D. A. Bryant and J. Zhou (GenBank genome NC_003488), and our survey of putative anion transporters indicated several potential candidates for another HCO_3^- transporter. Three genes in particular have been annotated as sulfate transporters because of a distant homology to plant sulfate transporters; they were initially referred to as *sul1*, *sul2*, and *sul3*. It was thought significant that *sul2* was located midway between the carboxysome-related operon (21) and genes encoding low-affinity CO_2 uptake (7, 22, 23) because it is speculated that some CO_2-concentrating mechanism-related gene clusters may have been transferred between cyanobacteria by lateral gene transfer (7). Accordingly, the *sul2* gene and its closest homolog, *sul3*, were insertionally inactivated, and the resulting strains were analyzed for defects in HCO_3^- uptake.

HCO_3^- uptake capacity was assessed at pH 9.3 as the response of photosynthetic net O_2 evolution to added Ci (Fig. 1). Here, it

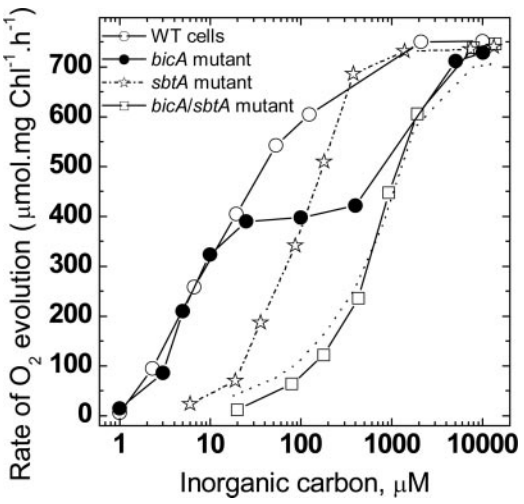

Fig. 1. The rate of photosynthetic O_2 evolution as a function of added Ci for *bicA* and *sbtA* knockout mutants of *Synechococcus* PCC7002 assayed at pH 9.3. Cells were grown at 2% CO_2 and transferred to CO_2-free air for 5 h. Typical responses are shown. The response of WT cells grown at 2% CO_2 is shown as a dashed line (no symbol).

is assumed that net uptake of Ci is 1:1 stoichiometric, with net O_2 evolution during steady-state photosynthesis. At pH 9.3, the CO_2 concentration at chemical equilibrium represents just 0.018% of total Ci, allowing the contribution of active CO_2 uptake to photosynthesis to be minimized relative to HCO_3^- uptake. Therefore, at this pH, the majority of photosynthetic O_2 evolution is related to active, net uptake of HCO_3^-, especially at Ci levels of <1 mM. Carbonate (CO_3^{2-}), the other Ci species at this pH, is not considered to be a substrate for active uptake, and there is no case in the literature for active CO_3^{2-} uptake.

Our analysis of the *sul3*/*sbtA* double mutant revealed that the *sul3* gene (hereafter as *bicA* for bicarbonate uptake) was found to be associated with expression of a low affinity HCO_3^- uptake activity (Fig. 1 and Table 1). At pH 9.3, the *sbtA*/*bicA* double mutant, exposed to CO_2-free air for 5 h (low-Ci cells), displayed a $K_{0.5}$(Ci) (the Ci concentration required for half maximal response) of nearly 820 μM, indicating a very low capacity for HCO_3^- uptake. WT cells grown at 2% CO_2 had a $K_{0.5}$(Ci) that was similar to the double mutant (Table 1 and dotted line in Fig. 1). By contrast, low-Ci WT cells displayed a $K_{0.5}$(Ci) of ≈18 μM,

Table 1. Photosynthetic affinities for Ci uptake in *Synechococcus* PCC7002 mutants determined as O_2 evolution responses at pH 9.3 (salt water media)

	High-Ci cells	Low-Ci cells	
Cell type	$K_{0.5}$(Ci), μM	$K_{0.5}$(Ci), μM	$K_{0.5}(HCO_3^-)$, μM*
WT PCC7002	720 ± 43	17.9 ± 0.4	6.5 ± 0.2
bicA (*sul3*) mutant	1,263 ± 84	5.1 ± 0.2†	1.8 ± 0.1
sbtA mutant	768 ± 29	107.0 ± 4.1	38.4 ± 1.5
bicA/*sbtA* mutant	1,960 ± 70	820.0 ± 51	295.0 ± 18
sul2 mutant	687 ± 25	18.5 ± 0.7	6.7 ± 0.3
sul2/*sbtA* mutant	700 ± 57	114.0 ± 11	41.2 ± 4.0

Data are shown as means ± SD ($n = 3$). Maximum photosynthetic rates were similar, with a mean of 846 μmol $O_2 \cdot$mg $Chl^{-1} \cdot h^{-1}$. Low-Ci cells were grown at 2% CO_2 and transferred to CO_2-free air for 5 h before assay.

*For calculation of $K_{0.5}(HCO_3^-)$, the $[HCO_3^-]$ at pH 9.3 was taken as 36% of total Ci species at chemical equilibrium.

†$K_{0.5}$(Ci) calculated for a maximum rate at 1 mM Ci (see Fig. 1).

PLANT BIOLOGY

implying a high photosynthetic affinity for HCO_3^-. In the *sbtA* mutant background, the BicA transporter displayed a $K_{0.5}$(Ci) of 107 μM, indicative of a transporter with relatively low affinity for HCO_3^-. Adjusted for the expected HCO_3^- concentration in salt-water medium, the BicA transporter would have a $K_{0.5}(HCO_3^-)$ of 38 μM (Table 1). Interestingly, the *bicA* insertional mutant revealed the kinetics of the SbtA transporter to be biphasic, with the first component showing a $K_{0.5}$(Ci) of 5 μM, saturating near 1 mM Ci (Fig. 1); the second component seems to be responsive to the action of the two CO_2 uptake systems, particularly at Ci >1 mM. Importantly, BicA is apparently capable of supporting a relatively high uptake flux for HCO_3^- because, at its intrinsic $K_{0.5}$(Ci), it supports 50% of the maximum photosynthetic rate, whereas SbtA supports ≈25% of the maximum rate (Fig. 1). Growth of the *bicA*, *sbtA*, and *sbtA*/*bicA* mutants at high CO_2 on plates and in liquid culture was indistinguishable from WT cells (data not shown). The mutants grew normally at pH 9.3 and 7.0 on plates in normal air (data not shown) due to a capacity for active CO_2 uptake to support growth. The mutants grew normally in liquid medium at pH 8.0 bubbled with normal air; however, the double mutant grew more slowly than WT at pH 9.3 (data not shown).

In high-Ci cells, there is evidence that BicA is expressed at low levels because a *bicA* mutant exhibits a $K_{0.5}$(Ci) that is greater than for WT cells (i.e., 1,263 vs. 720). Likewise, the SbtA transporter also may be active (Table 1). Consistent with this, *sbtA* and *bicA* mRNA were readily detected in high-Ci cells (data not shown). The closest homolog of *bicA*, namely *sul2*, does not seem to be associated with any appreciable HCO_3^- uptake activity because photosynthetic affinities for Ci in the *sul2* and *sul2*/*sbtA* mutants (low-Ci) were similar to low-Ci WT (Table 1).

CO_2-Responsive Expression of Genes Encoding Ci Transporters in *Synechococcus* PCC7002. The relative abundance of mRNA transcripts for *sul1*, *sul2*, *bicA*, and known Ci-responsive CO_2-concentrating mechanism genes (Ci transporters *sbtA* and *chpY*; transcriptional regulator *ndhR*), as well as a possible HCO_3^- porin that we have termed *porB*, was determined after Ci-limitation. High-Ci cells of exponentially growing *Synechococcus* PCC7002 (bubbled with 1.7% CO_2) were rapidly transferred to CO_2-free air for 3.5 h. First-strand cDNA was generated from normalized total RNA extracts, and quantitative real-time RT-PCR assays were performed by using gene-specific primers and *rnpA* and *petB* as reference genes (Fig. 8, which is published as supporting information on the PNAS web site).

Consistent with our data that the *bicA* gene product is a HCO_3^- transporter, the expression of *bicA* mRNA was strongly CO_2-responsive (Fig. 2*A*), increasing by ≈2,000% within 15 min of Ci-limitation, but returning to near basal levels between 90 and 120 min after the transition to Ci-limitation. By contrast, the expression of genes related to *bicA*, namely *sul1* and *sul2*, was down-regulated under Ci-limitation, suggesting that these genes may encode transporters of nutrients other than Ci. As has been previously observed in *Synechococcus* PCC7942 or *Synechocystis* PCC6803, the abundance of *ndhR* mRNA was also strongly, but transiently, induced upon Ci-limitation whereas transcript levels for *sbtA*, *chpY*, and *porB* (slr0042) were strongly induced, but at more sustained levels (10, 24, 25).

Gain-of-Function Expression of *bicA* and Close Homologs in *Synechococcus* PCC7942. A gain-of-function approach (17) was used to test whether expression of *bicA* from a plasmid was sufficient to confer HCO_3^- uptake activity in *Synechococcus* PCC7942. This host is ideal for this purpose because cells grown at high CO_2 possess a very low capacity for HCO_3^- uptake at high pH (Fig. 3) and lack close homologs of *bicA*. In assays at pH 9.3, expression of *bicA*-7002 resulted in substantial capacity for photosynthetic Ci uptake, with a $K_{0.5}$(Ci) of ≈102 μM; this result would equate to $K_{0.5}(HCO_3^-)$ of

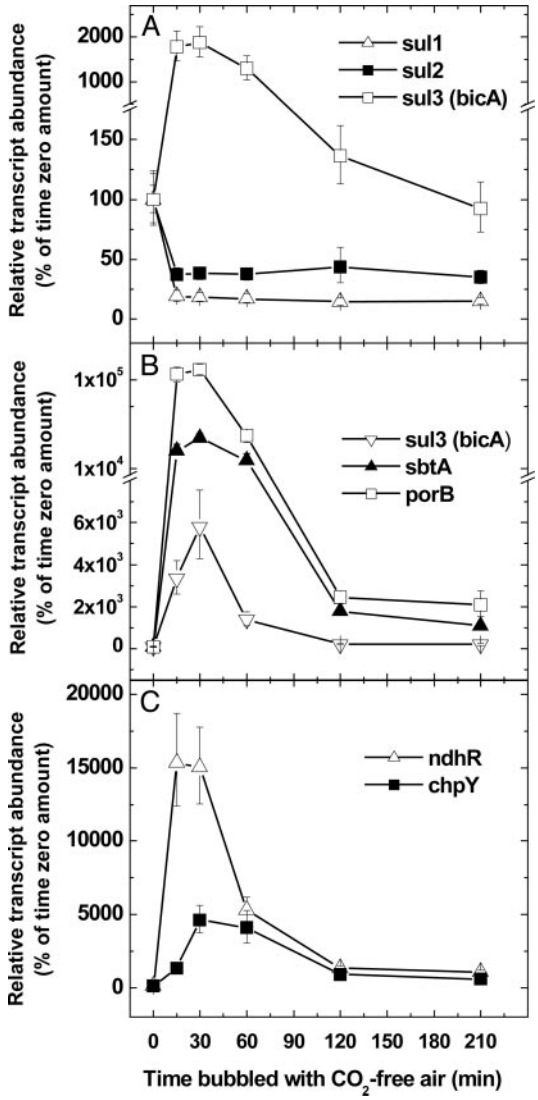

Fig. 2. CO_2-responsive expression of genes encoding putative Ci transporters and the transcriptional regulator *ndhR* in *Synechococcus* PCC7002 as determined by real-time RT-PCR. Symbols represent the extent of induction or repression after the shift to low Ci at each time point as a percentage of the high-Ci amount (set at 100%) + SE (n = 3). Representative results from two independent experiments are shown. Note *y*-axis break in *A* and *B*.

≈88 μM (Table 2 and Fig. 3). Clearly, expression of *bicA*-7002 gene product alone is sufficient to confer increased affinity and capacity for HCO_3^- uptake.

Expression of *bicA*-6803 in *Synechococcus* PCC7942 also led to increased photosynthetic Ci uptake activity, but with a lower affinity than for BicA-7002: $K_{0.5}$(Ci) of 198 μM and $K_{0.5}(HCO_3^-)$ of 171 μM (Table 2). Expression of *sul2*-7002 did not confer Ci uptake activity that differed from the WT response. Expression of *bicA*-WH led to a low level of Ci uptake activity (not shown) that was difficult to quantify in pH 9.3 assays (see below), but could be demonstrated at pH 8 in the presence of a CO_2 uptake inhibitor (Fig. 9, which is published as supporting information on the PNAS web site, and Table 5). BicA-7002 uptake activity was found to be Na^+-dependent because the photosynthetic response to Ci was stimulated by NaCl (Fig. 4) but unaffected by comparable additions of KCl (data not shown). Na^+ at 1.7 mM Na^+ (SD ± 0.1; n = 3) was required for half maximal stimulation; activity was maximal at ≈20 mM NaCl. The BicA-6803 transporter was also found to have

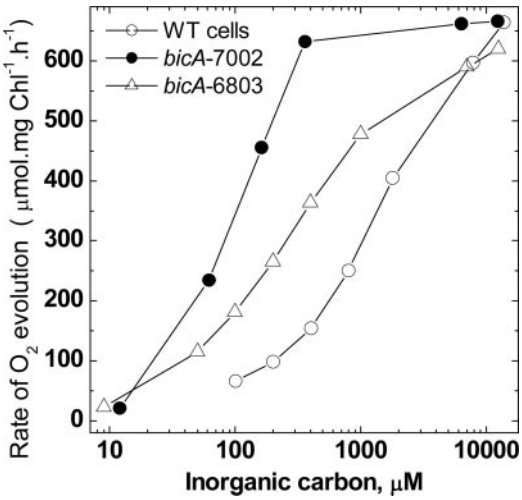

Fig. 3. The rate of photosynthetic O_2 evolution as a function of added Ci for cells of *Synechococcus* PCC7942 expressing *bicA*-7002 or *bicA*-6803 genes. Cells were grown at 2% CO_2 and assayed at pH 9.3. Typical responses are shown.

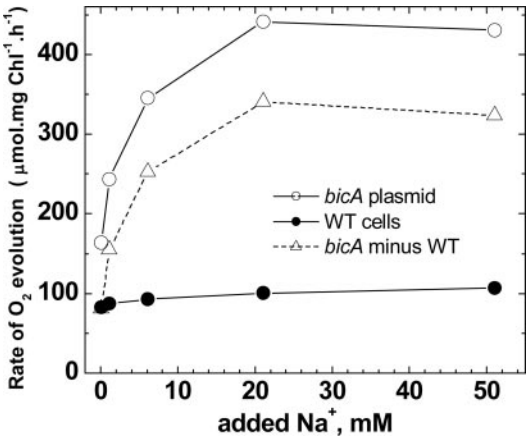

Fig. 4. Na^+-dependency of O_2 evolution due to *bicA*-7002 expression in *Synechococcus* PCC7942 (pH 9.3). Cells were grown at 2% CO_2 and washed in standard buffer with 0.1 mM Na^+ (pH 9.3). O_2 evolution was assayed at a Ci level of 270 μM $KHCO_3$. Typical responses are shown. BicA-7002 responses were subtracted from WT responses to yield a $C_{0.5}(Na^+)$ of 1.7 mM (SD = 0.1; $n = 3$).

a similar Na^+-dependence (data not shown). The response to Na^+ is similar to the Na^+-dependent SbtA transporter from *Synechocystis* PCC6803 (8). It is possible that BicA (or SbtA) are Na^+-driven (e.g., symport), but this conclusion cannot yet be drawn with confidence from any data presented in this paper, or previous experiments, in the case of SbtA (8).

Active Species Assays for HCO_3^- Uptake. Active species experiments for $H^{14}CO_3^-$ uptake (pH 9 conditions) were undertaken to further test our view that BicA is specific for HCO_3^- uptake. This view was supported. Experiments used short 15-s uptake periods after addition of $H^{14}CO_3^-$ (pH 9.5) followed by rapid termination with silicone-oil centrifugation. Such an approach measures initial rates of gross Ci uptake before chemical equilibrium between HCO_3^- and CO_2 is attained. Expression of *bicA*-7002 or *bicA*-6803 in *Synechococcus* PCC7942 led to enhanced rates of gross Ci uptake that approached substrate saturation around 1 mM Ci (Fig. 5*A*). This response is indicative of active uptake of HCO_3^- because the measured rates of uptake vastly exceeded the calculated maximum rate of CO_2 supply from HCO_3^- that could support CO_2 uptake (Fig. 5*A*, dotted line). By contrast, the Ci uptake response in WT cells (Fig. 5*A*), and cells expressing *sul2*-7002 (data not shown), was low and responded linearly to added Ci; these control rates also indicate a background capacity for HCO_3^- uptake because they were higher than the calculated rate of CO_2 supply (Fig. 5*A*). Interestingly, K_m (HCO_3^-) values (Fig. 5*B* and Table 3) were significantly higher than comparable $K_{0.5}(HCO_3^-)$ values inferred from steady-state photosynthetic measurements (Table 2). This finding might indicate that BicA is slow to respond to added HCO_3^- when using the short uptake approach; it was observed that HCO_3^--dependent O_2 evolution took 2–3 min to reach steady rates after each Ci increment (data not shown). Largely as expected, gross rates of initial HCO_3^- uptake exceeded estimates of net HCO_3^- uptake based on steady-state measurements (Fig. 3); reasons for this result in-

Table 2. Photosynthetic affinities for Ci uptake in cells of *Synechococcus* PCC7942 expressing *bicA* or *sul2* genes, determined as O_2 evolution responses at pH 9.3

Expression plasmid	$K_{0.5}$(Ci), μM	$K_{0.5}(HCO_3^-)$*, μM
No plasmid (WT cells)	1,185 ± 77	NA†
bicA-PCC7002	102 ± 10	88 ± 9
bicA-PCC6803	198 ± 17	171 ± 15
Sul2-PCC7002	1,100 ± 60	NA

Data are shown as means ± SD (n = 3–5). Maximum photosynthetic rates were similar, with a mean of 550 μmol O_2·mg Chl^{-1}·h^{-1}. Cells were grown at 2% CO_2 with 10 mM NH_4^+ in freshwater medium.

*For calculation of $K_{0.5}(HCO_3^-)$, the [HCO_3^-] at pH 9.3 was taken as 86.3% of total Ci species at chemical equilibrium.

†NA, not applicable.

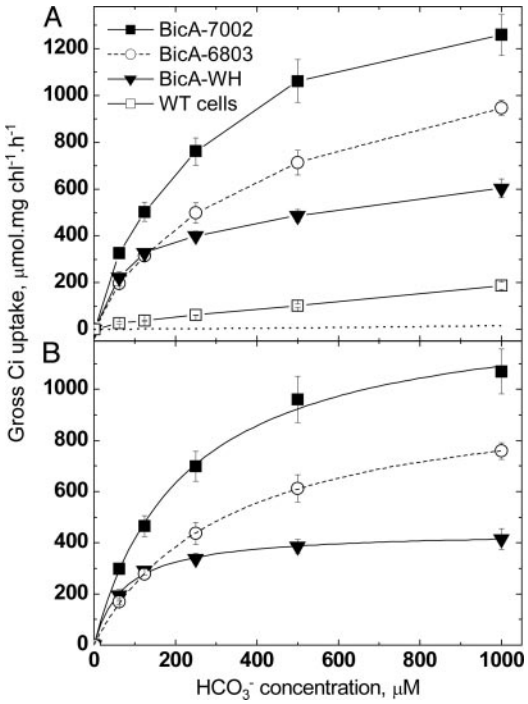

Fig. 5. Assessment of HCO_3^- uptake capacity by using silicone oil filtration. (*A*) Rates of gross ^{14}Ci uptake for *bicA* expression in *Synechococcus* PCC7942. Cells were grown at 2% CO_2 and assayed at pH 9 by using silicone oil filtration; 15-s uptakes were extrapolated to an hourly basis. Data are shown as mean ± SD (n = 3). The calculated maximum rate of CO_2 supply from HCO_3^- is plotted as a dotted line. (*B*) Data for *bicA* responses replotted after subtraction of WT response and fitted to Michaelis–Menton functions.

PLANT BIOLOGY

Table 3. HCO_3^- uptake data for cells of *Synechococcus* PCC7942 expressing *bicA* genes, determined by short-term uptake of $H^{14}CO_3^-$ at pH 9 using silicone-oil-filtration

Expression plasmid	$K_m(HCO_3^-)$, μM, for 2% CO_2 cells	V_{max} (μmol·mg $Chl^{-1}\cdot h^{-1}$)
No plasmid (WT)	NA*	NA
bicA-PCC7002	217 ± 23	1,333 ± 148
bicA-PCC6803	353 ± 29	1,013 ± 73
bicA-WH8102	75 ± 4	446 ± 36
Sul2-PCC7002	NA	NA

Uptake periods of 15 s were employed. Michaelis–Menton parameters were obtained by fitting curves to the data in Fig. 5*B*. Data are shown as means ± SD ($n = 3$); cells were grown at 2% CO_2.
*NA, not applicable for background rates of HCO_3^- uptake.

clude low ^{14}Ci leakage and lack of feedback regulation during the initial uptake period. For cells washed with 0.1 mM Na^+, it was found that HCO_3^- uptake in these assays due to BicA-7002 was stimulated by addition of 5 mM NaCl, but not by 5 mM KCl (data not shown). This finding indicates a potential role for Na^+ coupling in HCO_3^- uptake but also could indicate a role in cellular pH regulation.

It is of particular interest that expression of BicA-WH, from the oceanic strain *Synechococcus* WH8102, displayed HCO_3^- uptake activity with a V_{max} that was ≈33% of the BicA-7002 transporter (Fig. 5 and Table 3). Subtraction of the WT control response from the BicA-WH response yields uptake kinetics with a $K_m(HCO_3^-)$ of ≈75 μM. Given that this assay yields higher affinity estimates than steady-state assays, it is possible that BicA-WH may have a $K_{0.5}(HCO_3^-)$ that is significantly greater than 75 μM. There is also the possibility that expression of *bicA*-WH is not fully activated in the heterologous host. Nevertheless, the data at hand clearly indicate that the BicA homolog from *Synechococcus* WH8102 is capable of supporting a significant rate of HCO_3^- uptake.

Discussion

Bicarbonate Transport in *Synechococcus* PCC7002. Cyanobacteria living in oligotrophic ocean environments play a critical role in global CO_2 sequestration and primary photosynthetic productivity (1–3). However, relatively little is known about the genomics and physiology of active uptake processes for dissolved Ci, despite Ci uptake being the largest nutrient influx in these organisms. By contrast, linkages of specific gene products with physiological functions in active Ci uptake are well established in several freshwater cyanobacteria (4–6, 23).

In the present study we have identified BicA as a previously undiscovered class of Na^+-dependent HCO_3^- transporter that is functionally active in the coastal marine cyanobacterium, *Synechococcus* PCC7002. BicA is distinguishable as an extant member of the SulP family of anion transporters in eukaryotes and prokaryotes (9). We also have confirmed that the other HCO_3^- transporter present in *Synechococcus* PCC7002 is SbtA, an Na^+-dependent transporter with high affinity for HCO_3^-, first identified in *Synechocystis* (8). Together, BicA and SbtA account for most, if not all, of the HCO_3^- uptake capacity of low-CO_2 adapted *Synechococcus* PCC7002 cells at pH 9.3 (Fig. 1). Gain-of-function analyses (Figs. 3–5) demonstrated that the single *bicA*-7002 gene product is sufficient to support Na^+-dependent, HCO_3^- uptake in the freshwater cyanobacterium *Synechococcus* PCC7942, which lacks any close homologs of *bicA*. Analysis of gene transcripts in *Synechococcus* PCC7002 indicates that *bicA*, *sbtA*, and *porB* (coding for a putative porin) are highly upregulated under Ci limitation (Fig. 2); this finding is consistent with all three genes playing a role in active Ci uptake. Although BicA-7002 has a moderate photosynthetic uptake affinity for

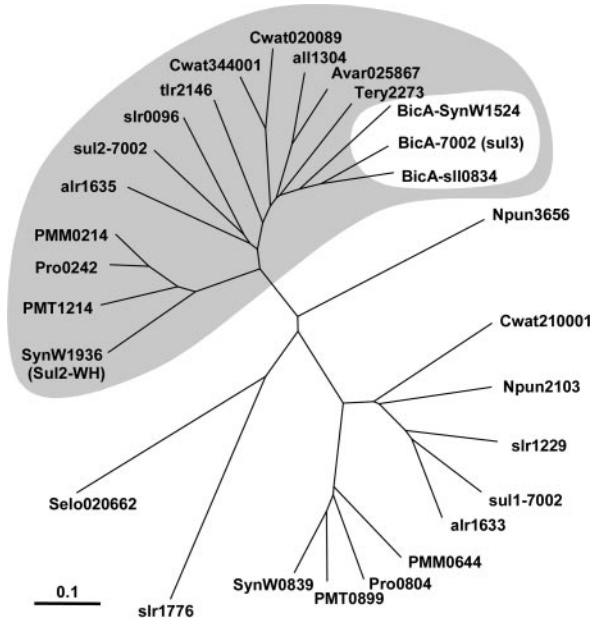

Fig. 6. Phylogenetic tree of sulfate transporter-like proteins in cyanobacteria. The CLUSTALW program was used to align the protein sequences, and the tree was generated in the TREEVIEW program. Abbreviations for species names: slr/sll, *Synechocystis* PCC6803; alr/all, *Anabaena* PCC7120; tlr/tll, *Thermosynechococcus* BP1; Npun, *Nostoc punctiforme*; Tery, *Trichodesmium erythraeum*; PMT, *Prochlorococcus marinus* MIT9313; PMM, *Prochlorococcus marinus* MED4; Pro, *Prochlorococcus marinus* SS120; SynW, *Synechococcus* WH8102; 7002, *Synechococcus* PCC7002; Cwat, *Crocosphaera watsonii* WH8501; Avar, *Anabaena variabilis* ATCC29413; and Selo, *Synechococcus elongatus* PCC7942. Marine species are represented by 7002, Tery, Cwat, SynW, Pro, PMT, and PMM.

HCO_3^- ($K_{0.5}$ of ≈38 μM), it is able to support a high photosynthetic flux rate, unlike the SbtA transporter, which supports a low flux rate with high uptake affinity ($K_{0.5} < 2$ μM). This pairing of complementary transport physiologies may be functionally significant in the natural environment. Indeed, the two HCO_3^- transporters acting together in fully induced WT cells are able to support a high flux rate with a net $K_{0.5}(HCO_3^-)$ of ≈6.5 μM (Table 1 and Fig. 1). This affinity for HCO_3^- is similar to that previously measured in *Synechococcus* PCC7002 (16).

BicA in Other Cyanobacteria. BicA proteins are predicted to be plasma membrane-targeted, possessing 8–12 membrane-spanning domains, and bioinformatics searches show them to be members of an extensive anion transporter family that is present in eukaryotes and prokaryotes. BicA appears to be the only prokaryotic member with a proven transport function. In prokaryotes, many of the proteins are presently annotated as sulfate transporters, but without accompanying experimental verification. BicA homologs are highly represented among the 13 cyanobacterial genomes that have so far been completely or partially sequenced. BicA homologs are present in some freshwater strains, but it is of particular interest to note that all of the marine cyanobacteria so far sequenced have one or more BicA homologs that cluster in lobe 1 of the phylogenetic tree shown in Fig. 6. In the present study, we have shown that a functional BicA homolog is present in at least one oceanic strain of cyanobacteria, namely *Synechococcus* WH8102. The rates of HCO_3^- uptake supported by this transporter seem to be relatively low compared with BicA-7002 (33% of BicA-7002 rate; Table 3); however, oceanic strains typically grow at about one doubling per day (or less), compared with *Synechococcus* PCC7002, which can support doubling times of 4–5 h. Thus, the low rates so far

demonstrated for the ectopically expressed BicA-WH transporter may be adequate to support typical rates of carbon gain in the euphotic zone where light intensities are typically 1–2% of full sunlight and carbon gain is light-limited. We speculate that BicA may act as a major route for Ci entry in oceanic cyanobacteria and thus have an important role in global CO_2 sequestration. This view requires further testing, despite the difficulties of culturing oceanic cyanobacteria in the laboratory.

Synechococcus WH7803 (related to WH8102) exhibits a net efflux of CO_2 under steady-state photosynthetic conditions (26), suggesting efficient HCO_3^- uptake but poor uptake of CO_2. A related study (27) found that low-CO_2 adapted cells of *Synechococcus* WH7803 have a good uptake affinity for Ci ($K_{0.5}$ of 13 μM) and showed rates of photosynthesis up to 70 μmol O_2·mg Chl^{-1}·h^{-1}. *Synechococcus* WH8102 possesses genes for low-affinity CO_2 uptake (7) and BicA-type HCO_3^- uptake (this study), but lacks genes for high-affinity CO_2 uptake and SbtA-type HCO_3^- uptake (7). By contrast, the *Prochlorococcus* species lack all known genes specific to either CO_2 uptake system, but possess a distant homolog of SbtA (7, 8) and close homologs of BicA (Fig. 6). It remains to be seen whether these distant homologs of SbtA are able to transport HCO_3^-, but here it is expected that the *Synechococcus* PCC7942 expression system will be useful. In summary, there is potential for BicA transporters to feature significantly in Ci uptake in oceanic *Synechococcus* spp. and *Prochlorococcus* spp. as well as other marine species such as *Crocosphaera watsonii* WH8501 and *Trichodesmium erythraeum* (Fig. 6).

It is interesting that the BicA homolog from *Synechocystis* PCC6803 transports HCO_3^- when expressed in *Synechococcus* PCC7942. This finding implies that BicA-6803 is an active transporter in *Synechocystis*. It is known that the gene is not up-regulated under Ci limitation (24), except for a modest up-regulation in an *ndhR* regulatory mutant, implying that the gene is normally constitutively expressed. This result would be consistent with the observation that high-CO_2 grown cells possess constitutive HCO_3^- uptake (28). Other physiological evidence (see figure 4C of ref. 8) is consistent with the presence of significant HCO_3^- uptake capacity in a *sbtA/cmpA* deletion mutant (lacking two known HCO_3^- transporters) of *Synechocystis* PCC6803, although this result was interpreted initially as periplasmic conversion of HCO_3^- to CO_2, followed by CO_2 uptake.

Wider Implications. Eukaryotic members of the SulP family have quite diverse functions, such as H^+/sulfate symport activity in plants and anion exchange in mammals, with different members showing different specificities, e.g., HCO_3^-/Cl^- exchange in kidney cells; some members have been implicated in human genetic diseases, such as Pendred syndrome, diastrophic dysplasia, and congenital chloride diarrhea (29). We have identified a previously unknown transport function for this family by showing that some members of the bacterial sulP family can actively transport HCO_3^- (possibly as Na^+/HCO_3^- symport); however, given the diversity of functions in eukaryotic members, it is reasonable to expect that the sulP family in prokaryotes can transport or exchange a number of inorganic anions such as sulfate, nitrate, and chloride. There is also scope to identify other sulP members that transport or exchange HCO_3^-, and it is interesting to note that close homologs of BicA exist in several bacteria. For instance, *Vibrio parahaemolyticus* (and related *Vibrio* species) has a homolog that shares 59% identity (over a span of 538 aa) with BicA-7002. This level of homology is similar to the identity between BicA-7002 and BicA-WH (namely 66% over 551 aa), suggesting that *Vibrio* homologs could act as HCO_3^- transporters with potential roles in HCO_3^- exchange/pH regulation or provision of HCO_3^- for anaplerotic metabolism. There is also a BicA homolog in the photosynthetic bacterium *Rhodospirillum rubrum* (47% identity over 545 aa).

Another interesting class of BicA homologs exists in several bacteria, namely *Streptomyces coelicolor*, *Streptomyces avermitilis*, *Leptospira interrogans*, *Pirellula* sp, *Mycobacterium tuberculosis*, and *Cytophaga hutchinsonii*. These bacteria possess homologs that are only 27–29% identical to BicA-7002, but are intriguing in that they possess a C-terminal fusion for a beta carbonic anhydrase protein involved in catalyzed interconversion of CO_2 and HCO_3^- species. This carbonic anhydrase fusion would make logical sense if the transporter domain of the protein were able to transport HCO_3^-. The physiological function of any such HCO_3^- transporter remains unclear at this stage.

We thank Ms. B. Dixon for expert technical assistance. Transcript analyses were supported with funding from an Australian Research Council Discovery Grant (to G.D.P.).

1. Field, C. B., Behrenfeld, M. J., Randerson, J. T. & Falkowski, P. (1998) *Science* **281,** 237–240.
2. Liu, H. B., Nolla, H. A. & Campbell, L. (1997) *Aquat. Microb. Ecol.* **12,** 39–47.
3. Partensky, F., Hess, W. R. & Vaulot, D. (1999) *Microbiol. Mol. Biol. Rev.* **63,** 106–127.
4. Kaplan, A. & Reinhold, L. (1999) *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **50,** 539–570.
5. Price, G. D., Maeda, S., Omata, T. & Badger, M. R. (2002) *Funct. Plant Biol.* **29,** 131–149.
6. Badger, M. R. & Price, G. D. (2003) *J. Exp. Botany* **54,** 609–622.
7. Badger, M. R., Hanson, D. & Price, G. D. (2002) *Funct. Plant Biol.* **29,** 161–173.
8. Shibata, M., Katoh, H., Sonoda, M., Ohkawa, H., Shimoyama, M., Fukuzawa, H., Kaplan, A. & Ogawa, T. (2002) *J. Biol. Chem.* **277,** 18658–18664.
9. Saier, M. H. (2000) *Microbiol. Mol. Biol. Rev.* **64,** 354–411.
10. Woodger, F. J., Badger, M. R. & Price, G. D. (2003) *Plant Physiol.* **133,** 2069–2080.
11. Klughammer, B., Sultemeyer, D., Badger, M. R. & Price, G. D. (1999) *Mol. Microbiol.* **32,** 1305–1315.
12. Dzelzkalns, V. A., Owens, G. C. & Bogorad, L. (1984) *Nucleic Acids Res.* **12,** 8917–8925.
13. Elhai, J. & Wolk, C. P. (1988) *Gene* **68,** 119–138.
14. Maeda, S., Kawaguchi, Y., Ohe, T. & Omata, T. (1998) *J. Bacteriol.* **180,** 4080–4088.
15. Price, G. D. & Badger, M. R. (1989) *Plant Physiol.* **91,** 505–513.
16. Sültemeyer, D., Price, G. D., Yu, J. W. & Badger, M. R. (1995) *Planta* **197,** 597–607.
17. Omata, T., Price, G. D., Badger, M. R., Okamura, M., Gohta, S. & Ogawa, T. (1999) *Proc. Natl. Acad. Sci. USA* **96,** 13571–13576.
18. Price, G. D. & Badger, M. R. (1989) *Plant Physiol.* **89,** 37–43.
19. Badger, M. R., Palmqvist, K. & Yu, J. W. (1994) *Physiol. Plant* **90,** 529–536.
20. Liu, W. H. & Saint, D. A. (2002) *Biochem. Biophys. Res. Commun.* **294,** 347–353.
21. Ludwig, M., Sultemeyer, D. & Price, G. D. (2000) *J. Phycol.* **36,** 1109–1118.
22. Maeda, S., Badger, M. R. & Price, G. D. (2002) *Mol. Microbiol.* **43,** 425–435.
23. Shibata, M., Ohkawa, H., Katoh, H., Shimoyama, M. & Ogawa, T. (2002) *Funct. Plant Biol.* **29,** 123–129.
24. Wang, H. L., Postier, B. L. & Burnap, R. L. (2004) *J. Biol. Chem.* **279,** 5739–5751.
25. McGinn, P. J., Price, G. D., Maleszka, R. & Badger, M. R. (2003) *Plant Physiol.* **132,** 218–229.
26. Tchernov, D., Hassidim, M., Vardi, A., Luz, B., Sukenik, A., Reinhold, L. & Kaplan, A. (1998) *Can. J. Bot.* **76,** 949–953.
27. Hassidim, M., Keren, N., Ohad, I., Reinhold, L. & Kaplan, A. (1997) *J. Phycol.* **33,** 811–817.
28. Benschop, J. J., Badger, M. R. & Price, G. D. (2003) *Photosynth. Res.* **77,** 117–126.
29. Mount, D. B. & Romero, M. F. (2004) *Eur. J. Physiol.* **447,** 710–721.