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The Carbon-Concentrating Mechanism of the Hydrothermal Vent Chemolithoautotroph *Thiomicrospira crunogena*

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Chemolithoautotrophic bacteria grow in habitats with a variety of dissolved inorganic carbon (DIC) concentrations and are likely to have transport-related adaptations to DIC scarcity. Carbon-concentrating mechanisms (CCMs) are present in many species of cyanobacteria, enabling them to grow in the presence of low concentrations of CO_2 by utilizing bicarbonate transporters and CO_2 traps to generate high intracellular concentrations of DIC. Similar CCMs may also be present in many other autotrophic bacteria. The sulfur-oxidizing γ-proteobacterial chemolithoautotroph *Thiomicrospira crunogena* experiences broad fluctuations in DIC availability at its hydrothermal vent habitat and may use a CCM to facilitate growth during periods of CO_2 scarcity. *T. crunogena* was cultivated in chemostats under DIC limitation to determine whether it has a CCM. Its K_{DIC} for growth was 0.22 mM, with a maximum growth rate of 0.44 h^{-1}. In short-term incubations with [^{14}C]DIC, DIC-limited cells had higher affinities for DIC (0.026 mM) than DIC-sufficient cells (0.66 mM). DIC-limited cells demonstrated an ability to use both extracellular CO_2 and HCO_3^-, as assayed by isotopic disequilibrium incubations. These cells also accumulated intracellular DIC to concentrations 100× higher than extracellular, as determined using the silicone oil centrifugation technique. Cells that were not provided with an electron donor did not have elevated intracellular DIC concentrations. The inducible changes in whole-cell affinity for DIC, the ability to use both extracellular CO_2 and HCO_3^-, and the energy-dependent generation of elevated intracellular concentrations of DIC are all consistent with the presence of a CCM in *T. crunogena*.

Some 2.7 billion years ago, this planet was geochemically, ecologically, and biologically distinct from what it is today. Atmospheric carbon dioxide concentrations were 1 to 3 orders of magnitude higher (16, 30). Representatives from all three domains of life (*Archaea*, *Bacteria*, and *Eukarya*) were already present and had begun to diversify (3). Confronted with the precipitous fall of atmospheric and oceanic CO_2 concentrations in the late Proterozoic, many autotrophic lineages likely responded with adaptations to maintain an adequate supply of CO_2 for growth (27, 35).

Carbon-concentrating mechanisms (CCMs) can facilitate rapid autotrophic growth in environments where the CO_2 and/or HCO_3^- concentrations are chronically or episodically low. CCMs are present in many species of cyanobacteria and generate an elevated intracellular concentration of HCO_3^- by using active HCO_3^- transport (22, 32) and CO_2 traps (33). Carboxysomal carbonic anhydrase (EC 4.2.1.1) converts intracellular HCO_3^- to CO_2, which is fixed by ribulose 1,5-bisphosphate carboxylase/oxygenase (Rubisco; EC 4.1.1.39) (2, 15, 26). The elevated intracellular concentrations of dissolved inorganic carbon (DIC) resulting from active transport expedite carbon fixation by Rubisco by enhancing substrate availability and mitigating the Rubisco oxygenase reaction (15).

CCMs have not been rigorously studied for any other prokaryotic autotrophs (e.g., autotrophic *Proteobacteria*, planktomycetes, green sulfur bacteria, *Aquificales*, and *Archaea*). This is surprising, as CCMs are likely to be quite relevant to primary productivity in the diverse habitats where autotrophic microorganisms are found. CCMs with similarities to those present in cyanobacteria may be widespread. For example, the recently sequenced genomes of the photosynthetic α-proteobacterium *Rhodopseudomonas palustris* and the ammonia-oxidizing β-proteobacterium *Nitrosomonas europaea* contain genes for carbonic anhydrase and potential HCO_3^- transporters (4, 18). Furthermore, it has recently been demonstrated that carboxysomes from the chemolithoautotroph *Halothiobacillus neapolitanus* contain carbonic anhydrase and are believed to function similarly to those present in cyanobacteria (34).

A CCM could facilitate the growth of chemolithoautotrophs at hydrothermal vents, where there is an enormous degree of spatial and temporal variability in the concentration of CO_2 (9). The hydrothermal vent γ-proteobacterium *Thiomicrospira crunogena* is an obligate sulfur-oxidizing chemoautotroph that was originally isolated from a deep-sea hydrothermal vent habitat where the CO_2 concentration oscillates between 20 μM and 1 mM, and HCO_3^- is always the most abundant form of DIC (equal to the sum of CO_2, HCO_3^-, and CO_3^{2-}) (9, 11). This is one of the fastest-growing chemoautotrophs, with a doubling time as low as 1 h (11). It continues to grow rapidly in batch culture even after drawing the concentration of DIC down to less than 20 μM, and it has carboxysomes (31). Both characteristics are consistent with the presence of a CCM.

Detailed physiological experiments were undertaken to determine whether this organism has a carbon-concentrating mechanism. Understanding how *T. crunogena* and other autotrophic microorganisms adapt to low concentrations of DIC during growth is relevant to understanding the physiology of these unique organisms and will provide insights into the response of autotrophic carbon fixation to changes in global geochemistry over Earth history.

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MATERIALS AND METHODS

Analytical methods and reagents. DIC (CO_2 plus HCO_3^- plus CO_3^{2-}) was quantified with an Agilent 6890N gas chromatograph equipped with an extractor

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to permit stripping dissolved gases from aqueous samples (5). Ammonia (NH_3 plus NH_4^+) was assayed using a commercial colorimetric kit (Sigma Inc.). After sonicating the cells for 30 s in the presence of glass beads, total protein was measured with a Lowry-type assay (Bio-Rad Inc.).

The [^{14}C]DIC used to measure carbon uptake and fixation rates was purchased as a sterile pH 9.5 solution (2 mCi ml^{-1}, 50 mM DIC; MP Biomedicals Inc.). Upon receipt, 0.5-ml portions were sealed into glass vials with gas-tight gas chromatograph septa and stored at 4°C until use. These stock [^{14}C]DIC solutions had stable counts over the course of this study (unpublished data).

Bacterial strains and growth conditions. *Thiomicrospira crunogena* XCL-2 (1) was cultivated at 25°C on liquid and solid TASW media modified from reference 11. TASW medium consists of artificial seawater supplemented with 40 mM thiosulfate, which *T. crunogena* utilizes as an electron donor, and Na HEPES to maintain the pH at 8 (100 mM in batch culture and 10 mM for continuous culture). The strain was maintained long term in 15% (vol/vol) glycerol-TASW medium at −80°C.

Cultivation under nutrient limitation. *T. crunogena* was cultivated in chemostats (New Brunswick Scientific BioFlo 110) to grow the cells under DIC or ammonia limitation. dO_2/pH controllers monitored the pH and O_2 concentrations in the growth chambers with electrodes, maintaining optimal growth conditions by adding 10 N KOH to keep the pH between 7.8 and 8 and by periodically pulsing the growth chamber with O_2 gas to maintain its concentration between 3 and 25 μM. The growth chamber was supplied with TASW medium [2.5 mM DIC, 6.6 mM $(NH_4)_2SO_4$] from a 10-liter reservoir at a range of dilution rates (0.03 to 0.44 h^{-1}).

Measurement of inducible changes in the half-saturation constant for DIC. To determine whether *T. crunogena* has inducible adaptations to cope with lower concentrations of DIC during growth, DIC-limited and DIC-sufficient (but ammonia limited) cells were cultivated in chemostats at a dilution rate of 0.1 vessel volume h^{-1} and their whole-cell affinities for DIC were measured. For DIC-sufficient cells, the reservoir [DIC] was raised to 10 mM, and the ammonia concentration was dropped from 13.2 mM to 0.5 mM. When the dilution rate was 0.1 vessel volume h^{-1}, the steady-state [DIC] in the growth chamber was 0.08 mM for DIC-limited cells and 5.5 mM for DIC-sufficient cells. The growth chamber ammonia concentration for DIC-sufficient cells was below the limit of detection for the assay used (<10 μM). DIC or ammonia was confirmed to be limiting growth by observing higher biomass densities in the growth chamber, assayed as protein concentrations, when either DIC or ammonia concentrations (as appropriate) were raised.

To harvest the cells, 150-ml portions were removed from the growth chamber and centrifuged (5,000 × *g*, 4°C, 10 min). Pellets were resuspended in 3 ml TASW medium (for DIC-limited cells, trace DIC, 13.2 mM ammonia; for ammonia-limited cells, 5.5 mM DIC and 0.5 mM ammonia) and kept on ice until the experiment was completed (less than 30 min).

Ten-microliter aliquots of the suspended cells were added to seven glass reaction vials with stir bars, filled with 1.98 ml TASW medium (pH 8, 0.02 to 10 mM DIC, supplemented with [^{14}C]DIC to a specific activity of 2 to 40 Ci/mol). Once per minute, over a time course of 4 min, a 400-μl aliquot was removed from each reaction vial and injected into a scintillation vial containing 200 μl 65°C glacial acetic acid. These acidified samples were gently sparged with air until dry to remove the [^{14}C]DIC. Scintillation cocktail was added to quantify the organic ^{14}C. Initial activities were measured by injecting 10-μl portions of the incubations into scintillation vials containing 3 ml scintillation cocktail plus 50 μl β-phenethylamine.

Bicarbonate and carbon dioxide uptake and fixation. To determine whether DIC-limited cells can use both extracellular bicarbonate and carbon dioxide, DIC-limited cells were cultivated and harvested as described above and resuspended in DIC-free TASW medium and bubbled with soda lime-treated (carbon dioxide-free) air until [DIC] = 0. Cell suspensions were then placed on ice and gently sparged with carbon dioxide-free air until use. Incubations with ^{14}C were conducted as described above, with the following modifications. Instead of using [^{14}C]DIC equilibrated at pH 8, an isotopic disequilibrium technique was used, in which either $H^{14}CO_3^-$ or $^{14}CO_2$ was added (6). The ^{14}C stock solution was pH 9.5 in distilled water, and therefore <0.1% CO_2, ~30% HCO_3^-, and 70% CO_3^{2-}. Upon injection into a well-buffered pH 8 solution, the HCO_3^- concentration instantaneously jumps to 93% due to protonation of most of the CO_3^{2-}. To prepare dissolved $^{14}CO_2$, ^{14}C stock solution was added to DIC-free 1 mM H_3PO_4 in a sealed glass vial and allowed to equilibrate for ~30 min before use to quantitatively convert the [^{14}C]DIC to $^{14}CO_2$.

Ten-microliter aliquots of suspended cells were added to reaction vials filled with 2 ml DIC-free TASW medium. $H^{14}CO_3^-$ or $^{14}CO_2$ was added to the vials to begin the reaction, and time points were taken at 10-s intervals. Upon addition, $^{14}CO_2$ generates $H^{14}CO_3^-$ with a half-dehydration time of ~17 s, while $^{14}CO_2$ forms from $H^{14}CO_3^-$ with a half-hydration time of 26 min (37). Accordingly, incubations in which $^{14}CO_2$ or $H^{14}CO_3^-$ was initially added were limited to 20 s or 40 s, respectively.

Intracellular DIC accumulation and pH. The silicone oil centrifugation method was used to measure the size of the intracellular DIC pool and the intracellular pH (modified from reference 14). DIC-limited cells were grown and harvested as described above. Eppendorf tubes (0.6 ml) were prepared containing two immiscible layers of fluid: a dense bottom layer, consisting of 20 μl of a killing solution (2:1 [vol/vol] 1 M glycine, pH 10, Triton) overlaid with 65 μl of silicone oil (Dow Chemicals SF 1156). Cell suspensions (200 μl) were pipetted on top of the silicone oil layer, mixed with radiolabeled solute, and, at timed intervals, were centrifuged for 20 s. The cells passed through the silicone layer and pelleted in the killing solution, carrying intracellular radiolabeled solute. Immediately after centrifugation, the tubes were frozen in liquid nitrogen and the bottom layer with the cells was clipped into scintillation vials containing 200 μl glacial acetic acid (for measuring intracellular fixed carbon) or directly into 3 ml scintillation cocktail plus 50 μl β-phenethylamine (for quantifying intracellular fixed and inorganic carbon, cell volumes, or pH).

For measuring intracellular DIC, TASW medium containing 3 to 240 μM [^{14}C]DIC (40 Ci mol^{-1}) was allowed to equilibrate at pH 8 before the experiment. Two hundred-microliter portions of this solution were pipetted on top of the silicone layers in Eppendorf tubes, and 10 μl of suspended cells in DIC-free TASW medium was added. After 30 s, the tubes were spun at 14,000 × *g* for 20 s before freezing and processing, as preliminary time course experiments indicated that the intracellular DIC pool was constant after 20 s. For each concentration of DIC, samples were run in parallel: to measure intracellular DIC plus fixed carbon, pellets were clipped into scintillation cocktail alkalinized with β-phenethylamine to measure fixed carbon, they were clipped into glacial acetic acid. Values from the samples clipped into glacial acetic acid were subtracted from those clipped into alkalinized scintillation cocktail to calculate the intracellular DIC pool. For estimating intracellular volume, which is necessary for calculating intracellular solute concentrations, incubations were also conducted with 9 μCi ml^{-1} D-sorbitol (U-^{14}C; MP Biomedicals) and 3 μCi ml^{-1} tritiated water (Amersham Biosciences) (14, 29). The intracellular concentrations of both of these substances reached equilibrium before 2 min (unpublished data). Accordingly, incubations with sorbitol or tritium were terminated at 2 min by centrifugation.

To assess whether DIC accumulation was energy dependent or simply driven by intracellular alkalinization, the intracellular pH of DIC-limited cells was measured using the silicone oil centrifugation technique described above, with [^{14}C]methylamine hydrochloride (ME; MP Biomedicals Inc.). ME has a pK_a of 10.7 and accumulates inside cells as its positively charged, conjugate acid when the cytoplasmic pH is lower than the extracellular pH (29, 38). ME was added to cell suspensions (pH 8) to an activity of 0.45 μCi ml^{-1} and allowed to equilibrate for 3 min, which was sufficient time to reach equilibrium concentrations inside the cells (K. Scott, unpublished data). Parallel incubations with [^{14}C]sorbitol and tritiated water were conducted with the cell suspensions to make it possible to estimate the intracellular concentrations of the ME.

Due to concern that ME might be accumulating in the cells via uptake by ammonia permeases (20), intracellular pH was also measured with [2-^{14}C]dimethadione (DMO) (5,5-dimethyl-2,4-oxazolidinedione; American Radiolabeled Chemicals) (29, 38). DMO has a pK_a of 6.2 (29, 38) and accumulates inside cells as its negatively charged, conjugate base. Incubating cells with DMO under conditions where the extracellular pH is much more alkaline than the intracellular pH prevents DMO from accumulating within the cells. To raise intracellular DMO concentrations, the pH used for incubations with this compound was 7.3 instead of 8.0. A second series of incubations with ME were also conducted at pH 7.3.

Energy dependence of intracellular DIC accumulation. Cells were cultivated under DIC-limiting conditions as described above. To prepare them for these experiments, it was necessary to wash them four times with thiosulfate-free artificial seawater medium as this electron donor is present in their growth medium at a high concentration (40 mM). After the final wash, cells were resuspended in DIC-free, thiosulfate-free medium and put on ice while being gently bubbled with CO_2-free air. Silicone oil centrifugation experiments with radiolabeled DIC were conducted as described above, at a range of thiosulfate concentrations (0 to 1 mM).

RESULTS

Cultivation under DIC limitation. Steady-state exponential growth, confirmed by protein and DIC assays, occurred after ~6 liters of TASW medium had passed through the 1-liter

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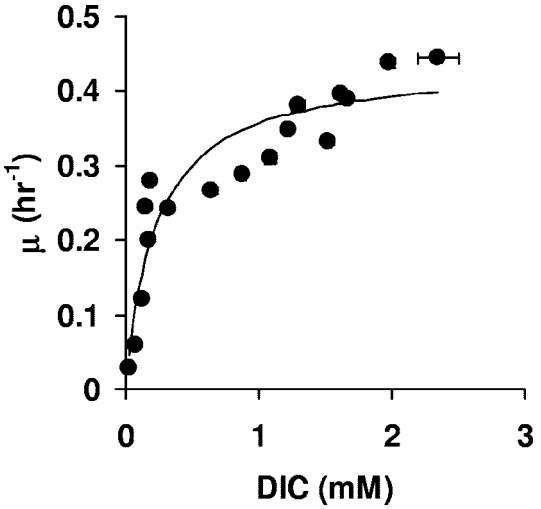

FIG. 1. Steady-state growth rate constants (μ) and DIC concentrations for *T. crunogena* cells grown in chemostats under DIC limitation. Using the direct linear plot method (8), a rectangular hyperbola was generated to model the data (shown as a curve in the figure; K_{DIC} = 0.22 mM; μ_{max} = 0.44 h^{-1}). The error bars indicate the standard error of the measurements (n = 3).

growth chamber. The steady-state DIC concentrations in the growth chamber were substantially lower than in the reservoir due to consumption by *T. crunogena* (Fig. 1). A rectangular hyperbola was fitted to the data via the direct linear plot method (8) to estimate the K_{DIC} (0.22 mM) and the maximum growth rate (μ_{max}) (0.44 h^{-1}).

Measurement of inducible changes in the half-saturation constant for DIC. DIC-limited cells had much higher whole-cell affinities for DIC (K_{DIC} = 0.026 mM) than DIC-sufficient cells did (K_{DIC} = 0.66 mM; Fig. 2), which is consistent with inducible changes in transport and/or fixation occurring when cells were growing under DIC-limiting conditions.

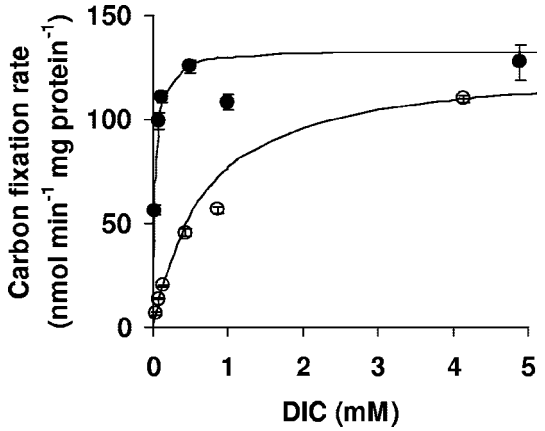

FIG. 2. Carbon fixation rates for *T. crunogena* harvested from DIC-limited (solid circles) and DIC-sufficient (ammonia limited; open circles) chemostats with μ = 0.1 h^{-1}. Data are presented with rectangular hyperbolae derived as in reference 8. For DIC-limited cells, the K_{DIC} (0.026 mM) was lower than for DIC-sufficient cells (K_{DIC} = 0.66 mM). The V_{max} values for both were similar (133 and 120 nmol min^{-1} mg $protein^{-1}$, respectively). The error bars indicate the standard deviations of the slopes.

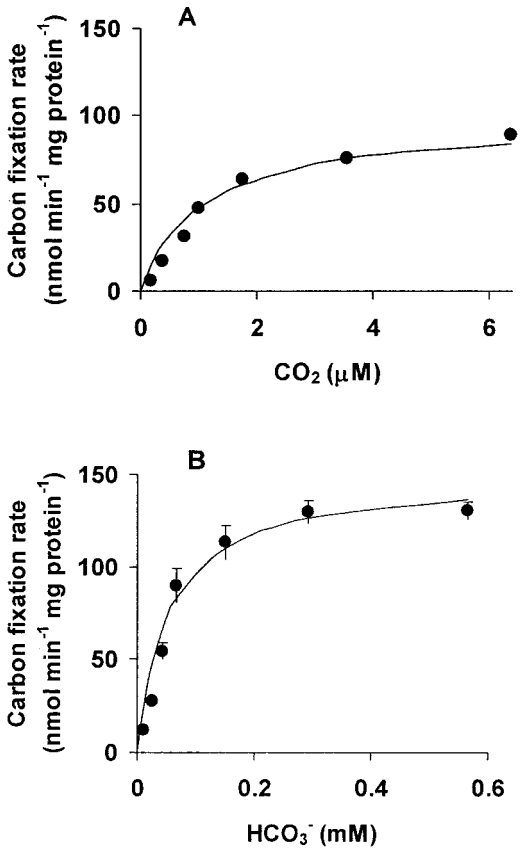

FIG. 3. Carbon fixation rates for *T. crunogena* harvested from DIC-limited chemostats with μ = 0.1 h^{-1}, when supplied with CO_2 (A) (K_{CO_2} = 1.03 μM; V_{max} = 97.2 nmol min^{-1} mg $protein^{-1}$) or HCO_3^- (B) ($K_{HCO_3^-}$ = 53.6 μM; V_{max} = 149 nmol min^{-1} mg $protein^{-1}$). Data are presented with rectangular hyperbolae derived as in reference 8. In panel B, error bars indicate the standard deviations of the slopes. Error bars are not presented in panel A since the carbon fixation rates were based on two time points.

Bicarbonate and carbon dioxide uptake and fixation. *T. crunogena* demonstrated an ability to use both extracellular carbon dioxide and bicarbonate (Fig. 3). Some interconversion of carbon dioxide and bicarbonate did occur over the time course of these experiments and needs to be considered when interpreting the results. When $^{14}CO_2$ was initially added, the $H^{14}CO_3^-$ formed was unlikely to contribute substantially to the observed carbon fixation rates, as the initial concentration of CO_2 was low (1 to 6 μM) relative to the $K_{HCO_3^-}$ (53.6 μM).

For the experiments in which $H^{14}CO_3^-$ was added, the $^{14}CO_2$ formed from $H^{14}CO_3^-$ over the 40-s time course probably contributed to the observed carbon fixation rates since the cells demonstrate such a high affinity for CO_2. To account for this, a pseudo-first-order rate constant for CO_2 formation from HCO_3^- in seawater was used to calculate the concentration of CO_2 present in the incubations at each time point (37). The contribution of this CO_2 to the measured rates of carbon fixation was estimated using the K_{CO_2} (1.03 μM) and V_{max} (97.2 nmol min^{-1} mg $protein^{-1}$) for CO_2-dependent carbon fixation. When this estimate of CO_2-dependent carbon fixation was subtracted from the rates measured in these experiments, the

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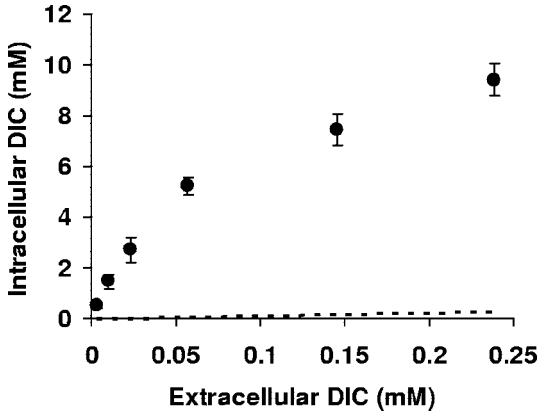

FIG. 4. Intracellular DIC accumulation by *T. crunogena* cultivated under DIC limitation in a chemostat (DIC = 0.1 mM, $\mu = 0.1\ h^{-1}$). A unity line (dashed; intracellular DIC = extracellular DIC) is presented with the data for comparison, and error bars are the standard errors of the intracellular concentrations ($n = 3$).

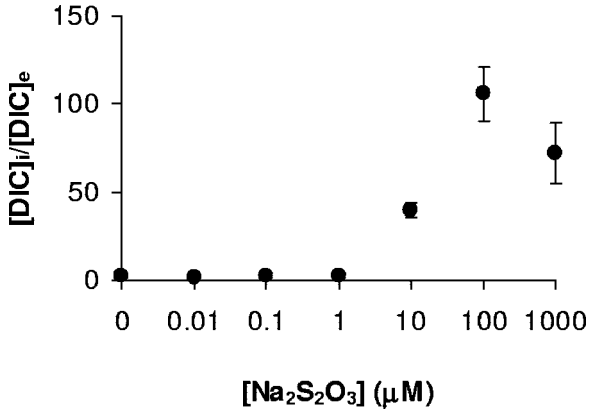

FIG. 5. Energy dependence of intracellular DIC accumulation by *T. crunogena*. Cells were cultivated under DIC limitation with thiosulfate ($Na_2S_2O_3$) as the electron donor. $[DIC]_i$ indicates the intracellular DIC concentration, $[DIC]_e$ indicates the extracellular DIC concentration (0.1 mM), and error bars indicate the standard error ($n = 3$).

$K_{HCO_3^-}$ was 11 μM and the V_{max} was 52 nmol min^{-1} mg $protein^{-1}$.

The data are consistent with bicarbonate use contributing substantially to growth under DIC limitation. At pH 8 and 80 μM DIC, the conditions under which these cells were grown, the bicarbonate concentration was 74 μM and the carbon dioxide concentration was ~0.7 μM (19). Using the K_s and V_{max} values (Fig. 3; parameters for HCO_3^- use corrected as described above) and the Michaelis-Menten equation, the carbon fixation rates due to bicarbonate and carbon dioxide use are 45 and 39 nmol min^{-1} mg $protein^{-1}$, respectively. Whether DIC-sufficient cells also demonstrate an ability to use extracellular bicarbonate remains to be determined; our manipulations of the DIC concentration in the cell suspensions to prepare them for these experiments would likely induce the expression of the same traits observed in DIC-limited cells.

Intracellular DIC accumulation and pH. Intracellular concentrations of DIC exceeded the extracellular concentration by 100× (Fig. 4), consistent with energy-dependent bicarbonate transport and accumulation in the cytoplasm. The intracellular pH of *T. crunogena* is ~7 at extracellular pHs of 8 (ME, 6.88 ± 0.10) and 7.3 (ME, 7.03 ± 0.06; DMO, 7.01 ± 0.32) (pH ± standard error; $n = 3$), precluding intracellular alkalinization as the driving force for intracellular DIC accumulation.

Energy dependence of intracellular DIC accumulation. Cells accumulated elevated intracellular concentrations of DIC when thiosulfate was present at concentrations greater than 1 μM (Fig. 5). This correlation of DIC accumulation and thiosulfate presence is consistent with intracellular DIC accumulation relying on either the membrane potential or ATP synthesis resulting from thiosulfate oxidation.

DISCUSSION

T. crunogena has a CCM that enables it to grow in the presence of low concentrations of CO_2 by generating an elevated concentration of intracellular DIC. The results presented here are consistent with active transport of bicarbonate or carbon dioxide playing a role. Active transport could create elevated intracellular DIC concentrations despite the intracellular pH being lower than the extracellular pH and would require the presence of thiosulfate or other electron donors for energy.

A CCM may serve to supplement the supply of CO_2 available at *T. crunogena*'s hydrothermal vent habitat. Temperature differences between bottom water (2°C) and dilute hydrothermal fluid (up to 35°C) create turbulent eddies at the vents. In turn, these eddies are responsible for seconds-to-hours-long oscillations of environmental CO_2 from 1 mM down to ~20 μM CO_2 (9, 12). During the lower-CO_2 periods, a CCM would enable *T. crunogena* to continue to grow rapidly.

The CCM present in *T. crunogena* has many parallels with cyanobacterial CCMs. In both cases, whole-cell affinities increase in response to the concentration of DIC available during growth. Energy-dependent transport generates an elevated concentration of intracellular DIC which, presumably in *T. crunogena* as in cyanobacteria, is "harvested" by carboxysomes (2, 26, 34). Use of extracellular HCO_3^- (Fig. 3) is consistent with the presence of HCO_3^- transporters, and a high cellular affinity for CO_2 (1 μM) compared to Rubisco (30 to 140 μM for form IA, 100 to 250 μM for form II) (10) may be indicative of an active CO_2 uptake system.

T. crunogena's genome (Scott, unpublished) encodes some genes whose products may function similarly to the components of cyanobacterial CCMs. An α-type carboxysome operon, which includes an ε-class carbonic anhydrase gene (34), is present. Additionally, a gene for a SulP-type anion transporter has been found, similar to the SulP-type transporters from marine cyanobacteria that have recently been demonstrated to have HCO_3^--transporting activity (24).

It is also likely that the *T. crunogena* CCM will have several features that distinguish it from cyanobacterial CCMs. In contrast to cyanobacteria, whose genomes have a single form IA or form IB Rubisco gene (13, 21, 23, 28), *T. crunogena*'s genome carries three genes for this enzyme (two form IA Rubisco genes and one form II Rubisco gene). The two form I Rubisco genes are expressed when cells are grown under low DIC conditions, while the form II Rubisco gene is preferentially

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expressed under high DIC conditions (Scott, unpublished), similar to what has been observed for *Hydrogenovibrio marinus* (36). Also different from cyanobacteria, *T. crunogena*'s genome lacks any apparent homologs for the cyanobacterial bicarbonate transporter genes *cmpABCD* (22) and *sbtA* (32), as well as *chpX* and *chpY*, which encode key components of the cyanobacterial CO_2 uptake system (25, 33). It is possible that novel HCO_3^- and CO_2 transporters will be found in this organism, and it will be interesting to determine whether transporter and Rubisco gene expression is coordinately regulated. The generation of random and directed knockout mutants is under way (K. P. Dobrinski and K. M. Scott, unpublished data), with the objective of deciphering the mechanism for intracellular DIC accumulation in this organism.

T. crunogena is not likely to be the only chemolithoautotroph with a CCM. CCMs may be present in other chemolithoautotrophs that have a high demand for DIC (e.g., due to rapid growth rates), utilize a sufficiently abundant or electronegative electron donor to offset the energetic burden of a CCM, and inhabit an environment with periods of DIC or CO_2 scarcity. Mechanistic and genetic studies of CCMs in several lineages of *Bacteria* and *Archaea* have the potential to illuminate how their ancestors coped with the 2-order-of-magnitude drop in CO_2 availability occurring over Earth history (16, 30).

Autotrophs may have addressed this dilemma with a single solution that was spread by horizontal gene transfer (as Rubisco genes were) (7). Alternatively, each lineage may have come up with a unique solution. It is reasonable to anticipate lineage-specific innovation, based on cyanobacterial CCMs. In cyanobacteria, three nonhomologous bicarbonate-transporting systems and two forms of carboxysomes are scattered among the different clades (2, 24). Other autotrophs inhabit microhabitats even more disparate than those where cyanobacteria flourish (17) and embrace an astounding degree of phylogenetic and physiological diversity (at least four divisions of *Bacteria* and *Archaea*). Perhaps this ecological and phylogenetic diversity is reflected in a genetic and mechanistic diversity of CCMs.

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REFERENCES

1. **Ahmad, A., J. P. Barry, and D. C. Nelson.** 1999. Phylogenetic affinity of a wide, vacuolate, nitrate-accumulating *Beggiatoa* sp. from Monterey Canyon, California, with *Thioploca* spp. Appl. Environ. Microbiol. **65:**270–277.
2. **Badger, M., D. Hanson, and G. D. Price.** 2002. Evolution and diversity of CO_2 concentrating mechanisms in cyanobacteria. Funct. Plant Biol. **29:**161–173.
3. **Brocks, J. J., G. A. Logan, R. Buick, and R. E. Summons.** 1999. Archean molecular fossils and the early rise of eukaryotes. Science **285:**1033–1036.
4. **Chain, P., J. Lamerdin, F. Larimer, W. Regala, V. Lao, M. Land, L. Hauser, A. Hooper, M. Klotz, J. Norton, L. Sayavedra-Soto, D. Arciero, N. Hommes, M. Whittaker, and D. Arp.** 2003. Complete genome sequence of the ammonia-oxidizing bacterium and obligate chemolithoautotroph *Nitrosomonas europaea*. J. Bacteriol. **185:**2759–2773.
5. **Childress, J. J.** 1984. Metabolic and blood characteristics in the hydrothermal vent tube-worm *Riftia pachyptila*. Mar. Biol. **83:**109–124.
6. **Cooper, T. G., and D. Filmer.** 1969. The active species of "CO_2" utilized by ribulose diphosphate carboxylase. J. Biol. Chem. **244:**1081–1083.
7. **Delwiche, C. F., and J. D. Palmer.** 1996. Rampant horizontal transfer and duplication of rubisco genes in eubacteria and plastids. Mol. Biol. **13:**873–882.
8. **Eisenthal, R., and A. Cornish-Bowden.** 1974. The direct linear plot. Biochem. J. **139:**715–720.
9. **Goffredi, S. K., J. J. Childress, N. T. Desaulniers, R. W. Lee, F. H. Lallier, and D. Hammond.** 1997. Inorganic carbon acquisition by the hydrothermal vent tubeworm *Riftia pachyptila* depends upon high external P-CO_2 and upon proton-equivalent ion transport by the worm. J. Exp. Biol. **200:**883–896.
10. **Horken, K., and F. R. Tabita.** 1999. Closely related form I ribulose bisphosphate carboxylase/oxygenase molecules that possess different CO_2/O_2 substrate specificities. Arch. Biochem. Biophys. **361:**183–194.
11. **Jannasch, H. W., C. O. Wirsen, D. C. Nelson, and L. A. Robertson.** 1985. *Thiomicrospira crunogena* sp. nov., a colorless, sulfur-oxidizing bacterium from a deep-sea hydrothermal vent. Int. J. Syst. Bacteriol. **35:**422–424.
12. **Johnson, K. S., J. J. Childress, and C. L. Beehler.** 1988. Short term temperature variability in the Rose Garden hydrothermal vent field. Deep-Sea Res. **35:**1711–1722.
13. **Kaneko, T., Y. Nakamura, C. P. Wolk, T. Kuritz, S. Sasamoto, A. Watanabe, M. Iriguchi, A. Ishikawa, K. Kawashima, T. Kimura, Y. Kishida, M. Kohara, M. Matsumoto, A. Matsuno, A. Muraki, N. Nakazaki, S. Shimpo, M. Sugimoto, M. Takazawa, M. Yamada, M. Yasuda, and S. Tabata.** 2001. Complete genomic sequence of the filamentous nitrogen-fixing cyanobacterium Anabaena sp. strain PCC 7120. DNA Res. **8:**205–213.
14. **Kaplan, A., M. R. Badger, and J. A. Berry.** 1980. Photosynthesis and the intracellular inorganic carbon pool in the bluegreen alga *Anabaena variabilis*: response to external CO_2 concentration. Planta **149:**219–226.
15. **Kaplan, A., R. Schwarz, J. Lieman-Hurwitz, M. Ronen-Tarazi, and L. Reinhold.** 1994. Physiological and molecular studies on the response of cyanobacteria to changes in the ambient inorganic carbon concentration, p. 469–485. *In* D. A. Bryant (ed.), The molecular biology of cyanobacteria. Kluwer Academic Publishers, Dordrecht, The Netherlands.
16. **Kaufman, A. J., and S. Xiao.** 2003. High CO_2 levels in the Proterozoic atmosphere estimated from analyses of individual microfossils. Nature **425:**279–282.
17. **Kuenen, J. G., and P. Bos.** 1989. Habitats and ecological niches of chemolitho(auto)trophic bacteria, p. 117–146. *In* H. G. Schlegel and B. Bowien (ed.), Autotrophic bacteria. Springer-Verlag, Madison, Wis.
18. **Larimer, F., P. Chain, L. Hauser, J. Lamerdin, S. Malfatti, L. Do, M. Land, D. Pelletier, J. Beatty, A. Lang, F. R. Tabita, J. L. Gibson, T. Hanson, C. Bobst, J. T. Y. Torres, C. Peres, F. Harrison, J. Gibson, and C. Harwood.** 2004. Complete genome sequence of the metabolically versatile photosynthetic bacterium *Rhodopseudomonas palustris*. Nat. Biotechnol. **22:**55–61.
19. **Lewis, E., and D. W. R. Wallace.** 1998. Program developed for CO_2 system calculations, ORNL/CDIAC-105. Oak Ridge National Laboratory, U.S. Department of Energy, Oak Ridge, Tenn.
20. **Muro-Pastor, M., J. C. Reyes, and F. J. Florencio.** 2005. Ammonium assimilation in cyanobacteria. Photosynth. Res. **83:**135–150.
21. **Nakamura, Y., T. Kaneko, S. Sato, M. Mimuro, H. Miyashita, T. Tsuchiya, S. Sasamoto, A. Watanabe, K. Kawashima, Y. Kishida, C. Kiyokawa, M. Kohara, M. Matsumoto, A. Matsuno, N. Nakazaki, S. Shimpo, C. Takeuchi, M. Yamada, and S. Tabata.** 2003. Complete genome structure of Gloeobacter violaceus PCC 7421, a cyanobacterium that lacks thylakoids. DNA Res. **10:**137–145.
22. **Omata, T., G. D. Price, M. R. Badger, M. Okamura, S. Gohta, and T. Ogawa.** 1999. Identification of an ATP-binding cassette transporter involved in bicarbonate uptake in the cyanobacterium *Synechococcus* sp. strain PCC 7942. Proc. Natl. Acad. Sci. USA **96:**13571–13576.
23. **Palenik, B., B. Brahamsha, F. W. Larimer, M. Land, L. Hauser, P. Chain, J. Lamerdin, W. Regala, E. Allen, J. McCarren, I. Paulsen, A. Dufresne, F. Partensky, E. Webb, and J. Waterbury.** 2003. The genome of a motile marine *Synechococcus*. Nature **424:**1037–1042.
24. **Price, G., F. Woodger, M. Badger, S. Howitt, and L. Tucker.** 2004. Identification of a SulP-type bicarbonate transporter in marine cyanobacteria. Proc. Natl. Acad. Sci. USA **101:**18228–18233.
25. **Price, G. D., S. Maeda, T. Omata, and M. R. Badger.** 2002. Modes of active inorganic carbon uptake in the cyanobacterium, Synechococcus sp. PCC7942. Funct. Plant Biol. **29:**131–149.
26. **Price, G. D., D. Sultemeyer, B. Klughammer, M. Ludwig, and M. Badger.** 1998. The functioning of the CO_2 concentrating mechanism in several cyanobacterial strains: a review of general physiological characteristics, genes, proteins, and recent advances. Can. J. Bot. **76:**973–1002.
27. **Raven, J. A.** 1991. Implications of inorganic carbon utilization: ecology, evolution, and geochemistry. Can. J. Bot. **69:**908–923.
28. **Rocap, G., F. W. Larimer, J. Lamerdin, S. Malfatti, P. Chain, N. A. Ahlgren, A. Arellano, M. Coleman, L. Hauser, W. R. Hess, Z. I. Johnson, M. Land, D. Lindell, A. F. Post, W. Regala, M. Shah, S. L. Shaw, C. Steglich, M. B. Sullivan, C. S. Ting, A. Tolonen, E. A. Webb, E. R. Zinser, and S. W. Chisholm.** 2003. Genome divergence in two Prochlorococcus ecotypes reflects oceanic niche differentiation. Nature **424:**1042–1047.
29. **Rottenberg, H.** 1979. The measurement of membrane potential and ΔpH in cells, organelles, and vesicles. Methods Enzymol. **55:**547–569.

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30. **Rye, R., P. H. Kuo, and H. D. Holland.** 1995. Atmospheric carbon dioxide concentrations before 2.2 billion years ago. Nature **378:**603–605.
31. **Scott, K. M., M. Bright, and C. R. Fisher.** 1998. The burden of independence: inorganic carbon utilization strategies of the sulphur chemoautotrophic hydrothermal vent isolate *Thiomicrospira crunogena* and the symbionts of hydrothermal vent and cold seep vestimentiferans. Cah. Biol. Mar. **39:** 379–381.
32. **Shibata, M., H. Katoh, M. Sonoda, H. Ohkawa, M. Shimoyama, H. Fukuzawa, A. Kaplan, and T. Ogawa.** 2002. Genes essential to sodium-dependent bicarbonate transport in cyanobacteria. J. Biol. Chem. **277:**18658–18664.
33. **Shibata, M., H. Ohkawa, H. Katoh, M. Shimoyama, and T. Ogawa.** 2002. Two CO_2 uptake systems in cyanobacteria: four systems for inorganic carbon acquisition in *Synechocystis* sp. strain PCC6803. Funct. Plant Biol. **29:**123–129.
34. **So, A. K.-C., G. S. Espie, E. B. Williams, J. M. Shively, S. Heinhorst, and G. C. Cannon.** 2004. A novel evolutionary lineage of carbonic anhydrase (ε class) is a component of the carboxysome shell. J. Bacteriol. **186:**623–630.
35. **Tortell, P. D.** 2000. Evolutionary and ecological perspectives on carbon acquisition in phytoplankton. Limnol. Oceanogr. **45:**744–750.
36. **Yoshizawa, Y., K. Toyoda, H. Arai, M. Ishii, and Y. Igarashi.** 2004. CO_2-responsive expression and gene organization of three ribulose-1,5-bisphosphate carboxylase/oxygenase enzymes and carboxysomes in *Hydrogenovibrio marinus* strain MH-110. J. Bacteriol. **186:**5685–5691.
37. **Zeebe, R. E., and D. Wolf-Gladrow.** 2003. CO_2 in seawater: equilibrium, kinetics, isotopes. Elsevier, New York, N.Y.
38. **Zilberstein, D., V. Agmon, S. Schuldiner, and E. Padan.** 1984. *Escherichia coli* intracellular pH, membrane potential, and cell growth. J. Bacteriol. **158:**246–252.

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