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Isolation and Characterization of a Mutant of *Chlamydomonas reinhardtii* Deficient in the CO_2 Concentrating Mechanism[1]

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ABSTRACT

A *Chlamydomonas reinhardtii* mutant has been isolated that cannot grow photoautotrophically on low CO_2 concentrations but can grow on elevated CO_2. In a test cross, the high CO_2-requirement for growth showed a 2:2 segregation. This mutant, designated *CIA-5*, had a phenotype similar to previously identified mutants that were defective in some aspect of CO_2 accumulation. Unlike previously isolated mutants, *CIA-5* did not have detectable levels of the periplasmic carbonic anhydrase, an inducible protein that participates in the acquisition of CO_2 by *C. reinhardtii*. *CIA-5* also did not accumulate inorganic carbon to levels higher than could be accounted for by diffusion. This mutant strain did not synthesize any of the four polypeptides preferentially made by wild type *C. reinhardtii* when switched from an environment containing elevated CO_2 levels to an environment low in CO_2. It is concluded that this mutant fails to induce the CO_2 concentrating system and is incapable of adapting to low CO_2 conditions.

Chlamydomonas reinhardtii, like other unicellular green algae, has the capacity to adapt to varying CO_2 concentrations in the environment (1–3, 5). When grown on elevated levels of CO_2 (5% (v/v) in air), *C. reinhardtii* has a relatively low affinity for C_i[2] and exhibits high rates of photorespiration when placed in a low CO_2/high O_2 environment (2, 16, 19, 26). However, if *C. reinhardtii* remains exposed to low levels of CO_2 (ambient CO_2 levels), it adapts to these conditions by inducing a CO_2 concentrating mechanism (2). This CO_2 concentrating mechanism is thought to increase the CO_2 at the active site of ribulose-1,5-bisphosphate carboxylase/oxygenase thereby increasing CO_2 fixation and reducing the competing oxygenase activity of rubisco to decrease photorespiration.

The mechanism by which *C. reinhardtii* accomplishes this CO_2 concentration involves several proteins. It is presently thought to involve a C_i transporter (14, 20) and one or more isozymes of carbonic anhydrase (10, 14, 21). A carbonic anhydrase located in the periplasmic space of the alga has been identified as part of the CO_2 concentrating system (6, 11, 31). This protein is made only when the cells are grown in the light with low concentrations of CO_2 (8, 27, 29, 30). The existence of other components of the CO_2 concentrating system has been inferred from studies of *C. reinhardtii* mutants (23–25), C_i uptake measurements (21), and from ^{35}S-labeling studies of cells that are adapting to low CO_2 conditions (13).

A number of *C. reinhardtii* strains have been isolated that appear to be defective in some aspect of CO_2 accumulation (18, 23–26). These mutants are capable of photoautotrophic growth, but only if the CO_2 concentration in the environment is elevated. If these strains are placed on low levels of CO_2, they either cannot grow, or grow at slower than normal rates. Some of these strains are unable to accumulate C_i (24), while others accumulate C_i to levels higher than wild-type cells (23), but are unable to use this accumulated C_i efficiently. All of the previously characterized *C. reinhardtii* mutants were capable of inducing the synthesis of the periplasmic carbonic anhydrase (18, 26) as indicated by enzyme assay or by detection with antibodies raised against the periplasmic carbonic anhydrase. A *Dunaliella* mutant missing the external carbonic anhydrase has been reported (4). This report describes the isolation of a *C. reinhardtii* strain, *CIA-5* that is missing the periplasmic carbonic anhydrase. $[^{14}C]HCO_3^-$ uptake studies and ^{35}S-labeling experiments indicate that other components of the CO_2 concentrating system are missing in this strain as well. It appears that *CIA-5* is incapable of forming any of the proteins associated with the C_i accumulating mechanism.

MATERIALS AND METHODS

Algal Culture Conditions

The wild-type strain, of *Chlamydomonas reinhardtii*, 137 mt^+, has been maintained in R. K. Togasaki's laboratory.

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[2] Abbreviations: C_i, inorganic carbon ($CO_2 + HCO_3^-$); EZ, ethoxzolamide; $K_{0.5}(CO_2)$, the CO_2 concentration at which the rate of CO_2-dependent photosynthetic O_2 evolution is half maximal; high CO_2, air supplemented with CO_2 so that the final CO_2 concentration is 5% (v/v); low CO_2, air containing ambient (340 ppm) CO_2 concentrations.

Strain CC-124 *mt*⁻, was from the Duke University *Chlamydomonas* Genetics Center. Wild-type and *CIA-5* were maintained on plates with yeast-acetate medium (28) until a few weeks before use. In liquid culture, cells were grown in minimal media (28) in 2.8 L flasks illuminated with 200 $\mu E \cdot m^{-2} \cdot s^{-1}$. These cultures were bubbled with air supplemented with 5% (v/v) CO_2 until they reached a density of 3 to $5 \cdot 10^6$ cells$\cdot ml^{-1}$ and then either switched to bubbling with air containing about 0.035% CO_2 or left on the high CO_2 gas regime.

For growth studies, both wild-type and *CIA-5* cells were precultured on Tris-acetate-phosphate (TAP) medium in 500 mL flask as described by Gorman and Levine (9). Cells were harvested during log phase growth by centrifugation at 3000*g* for 5 min, washed once in minimal media (28), and resuspended in minimal media to give an initial A_{750} between 0.03 and 0.05. One hundred mL suspensions of cells in 500 mL Nephelo culture flasks were illuminated on a rotary shaker (100 rpm) with precalibrated air/CO_2 mixtures (Air Products Co.) passed over the surface of the culture at 7 mL min^{-1}. The increase in A_{750} with time was used to estimate the growth rate.

Isolation of High CO_2-Requiring Mutants

High CO_2 requiring-mutants were isolated as previously described (7) except for the following modifications. Following mutagenesis by UV irradiation, an aliquot of the cell suspension was plated onto minimal medium, kept in the dark for 24 h, and then placed in a sealed plastic container containing 150 mL of a saturated $NaHCO_3$ solution in a beaker. These plates were illuminated (300 $\mu E \cdot m^{-2} \cdot s^{-1}$) for 3 d and the saturated bicarbonate solution was replaced with one of water. The box was then resealed for 10 to 14 days. At this point colonies that were very small were picked and transferred to plates containing TAP medium (9). These colonies were then tested for high CO_2 dependence for photoautotrophic growth as previously described (7). The final yield of high CO_2-requiring colonies was 0.1% of those colonies that survived UV irradiation. *CIA-5* was selected by this procedure and was isolate 8506-Y-11.

In the cross of *CIA-5* with CC-124, the induction of gametes, zygote maturation and tetrad dissection were performed as previously described (22). The resultant colonies were tested for the high CO_2 growth requirement, mating type, and in some cases the $K_{0.5}(CO_2)$.

Assays

Photosynthetic CO_2-dependent O_2 evolution was measured in a Rank Brothers O_2 electrode (2, 16). Cells were harvested and resuspended in the buffers indicated in the table and figure legends, at a concentration of 1 to 2 mg Chl$\cdot ml^{-1}$. This concentrated suspension was then diluted to 25 μg Chl ml^{-1} in the electrode chamber (4 mL total volume) and illuminated with 600 $\mu E\ m^{-2} \cdot s^{-1}$ of 400 to 700 nm light. The determination of the apparent affinity of the algal cells for CO_2 [$K_{0.5}(CO_2)$] was calculated as previously described (16, 17). Chl concentrations were determined spectrophotometrically after extraction with 80% acetone.

The accumulation of C_i by the algal cells was estimated by centrifugation through silicone oil as previously described (2, 3, 16). Assays were performed in the light (400 $\mu E \cdot m^{-2} \cdot s^{-1}$) at 25°C in 400 μL microfuge tubes in either a Beckman Microfuge 11 or a Microfuge B. The tubes contained (from bottom to top): 25 μL of 1 M glycine (pH 10) containing 0.75% SDS; 65 μL of silicone oil (1:1 [v/v] of Wäcker AR20 and Wäcker AR200]; and 280 μL of the algal suspension that had been depleted of C_i by illuminating the sample until O_2 evolution ceased. The uptake was initiated by the addition of $H^{14}CO_3^-$ into the algal suspension and terminated by a 15 s centrifugation. Inorganic carbon uptake by the cells was estimated from the difference between the total ^{14}C and the acid-stable ^{14}C in the pellet (2).

Carbonic anhydrase was measured in intact cells at 4°C by adding 100 μL of a cell suspension (250 μg Chl$\cdot mL^{-1}$) to 5 mL of 20 mM 4-(2-hydroxyethyl)-1-piperazinepropane sulfonic acid (Epps) pH 8.3, and the reaction was initiated by the addition of 3.4 mL of ice cold CO_2-saturated water. The time required for the pH to drop from 8.3 to 6.3 was measured and the activity of carbonic anhydrase calculated using the equation: units = $10[(t_0/t)-1]$ where t is the time measured for the pH change to occur when cells are present and t_0 is the time required for the pH change to occur when no cells are present. A second t_0 was measured which included cells but also contained a large excess of the carbonic anhydrase inhibitor acetzaolamide.

Extracts from wild-type or *CIA-5* cells were probed with antibodies prepared against the *C. reinhardtii* periplasmic carbonic anhydrase as described in the accompanying manuscript (10).

^{35}S-Labeling of Wild-Type and *CIA-5* Cells

Algal cells were labeled during the adaptation period essentially as described by Manuel and Moroney (13). Wild-type or *CIA-5* cells were grown on minimal media containing 1/5 the normal sulfate concentration for 1 to 2 d prior to the experiment. These cultures were maintained on high CO_2. For ^{35}S-labeling, cells were harvested and resuspended in minimal media lacking sulfate and bubbled either with high CO_2 (control cells) or switched to low levels of CO_2. $^{35}SO_4^{-2}$ (1000 Ci $mmol^{-1}$) was added and cells were illuminated for 3 h. The labeled cells were then harvested and washed with 50 mM Tris-HCl (pH 7.4) at 4°C, and extracted with chloroform:methanol (1:1 v/v). The resulting pellets were subjected to SDS-PAGE (12), and the labeled bands visualized by autoradiography on Kodak X-OMAT film.

Materials

Acetazolamide (5-acetamido-1,3,4-thiodiazole-2-sulfonamide) was from Sigma Chemical Company and EZ (6-ethoxy-2-benzathiazole-2-sulfonamide) was a gift from Dr. Thomas H. Maren. Wacker silicone oils were provided by SWS Silicones, Adrian, MI. $NaH^{14}CO_3$ was purchased from New England Nuclear and $H_2\ ^{35}SO_4$ was purchased from ICN.

RESULTS

Selection of the *CIA-5* Strain

CIA-5 was selected from a mutagenized *C. reinhardtii* population using the procedures outlined in "Materials and Methods." This strain was able to grow photoautotrophically only when the CO_2 concentration was significantly above ambient levels. When the CO_2 concentration was low, this strain grew much more slowly than wild-type cells (Table I). *CIA-5* could also grow photoheterotrophically on acetate at rates close to wild-type cells (data not shown). A test cross of *CIA-5* with a culture with wild type growth characteristics (CC-124), yielded a 2:2 segregation of the high CO_2 growth requirement (Table II). These results indicate that the high CO_2 growth requirement appears to be a single mutation that is inherited in a Mendelian fashion. Thus the mutation in *CIA-5* is a nuclear one.

In addition, these growth characteristics indicated that *CIA-5* may have a defect in the inorganic carbon accumulation system, as its phenotype is very similar to previously described strains (18, 23, 24). This hypothesis was supported by measurements of the affinity of *CIA-5* cells for CO_2 [$K_{0.5}(CO_2)$]. Unlike wild-type cells that adapt to low CO_2 conditions by increasing their apparent affinity for CO_2, *CIA-5* cells never showed this increased affinity for CO_2 even when they were grown on limiting CO_2 concentrations (Table III). The $K_{0.5}(CO_2)$ of high CO_2 or low CO_2-grown *CIA-5* cells was similar and high. The $K_{0.5}(CO_2)$ of the *CIA-5* cells never approached the low value seen in wild-type cells. Therefore inorganic carbon uptake and carbonic anhydrase levels were measured in this strain to further characterize the lesion present in *CIA-5*.

Table I. *Doubling Times for Wild-Type and CIA-5 Cells on Various CO_2 Concentrations in Air*

Cell suspensions in minimal medium were agitated under atmospheres of the indicated CO_2 concentrations. Growth rates were monitored turbidometrically at 750 nm

CO_2 Concentration	Doubling Time	
	Wild-type	*CIA-5*
%	*h*	
5.0	7	9
0.106	7	12
0.030	12	23

Table II. *Analysis of a Cross between C. reinhardtii Mutant CIA-5 mt^+ and Wild-Type Strain CC-124 mt^-*

Tetrad/Octad Progeny	Growth on Low CO_2[a]	Mating Type
2-1	Wt	+
2-2	Wt	−
2-3	Slow	+
2-4	Wt	+
2-5	Slow	−
2-6	Wt	−
2-7	Slow	+
2-8	Slow	−
4-1	Wt	+
4-2	Slow	−
4-3	Slow	−
4-4	Wt	+
8-1	Slow	+
8-2	Wt	−
8-3	Wt	−
8-4	Slow	+

[a] Wt, colony showed wild-type growth on low CO_2; slow, colony showed inhibited growth on low CO_2 but wild-type growth on elevated CO_2.

Carbonic Anhydrase Determinations

The levels of the periplasmic carbonic anhydrase present in *CIA-5* were much lower than wild-type cells (Table IV). A minor amount of carbonic anhydrase activity was detected, but these levels represent less than 5% of the amounts found in wild-type cells. Immunoblots of extracts of wild-type and *CIA-5* cells were performed to determine whether the periplasmic carbonic anhydrase was present in *CIA-5* cells. These assays failed to detect any periplasmic carbonic anhydrase in either high CO_2 grown or low CO_2 grown cultures of *CIA-5* (Fig. 1). On the other hand, this protein was easily detected in low CO_2 grown cultures of wild-type cells (Fig. 1). These data indicate that *CIA-5* cells had little or no periplasmic carbonic anhydrase, which makes this strain different from other high CO_2-requiring *C. reinhardtii* mutants previously isolated, all of which had levels similar to that found in wild-type cells.

Table III. *Photosynthetic $K_{0.5}(CO_2)$ Values for Wild-Type and CIA-5 Cells*

Wild-type or *CIA-5* cells were grown with high concentrations of CO_2 or with air as indicated. The $K_{0.5}(CO_2)$ determinations were done in either 25 mM Hepes-KOH (pH 7.3) or 25 mM K-citrate (pH 5.1) at cell concentrations of 25 μg Chl·ml^{-1}.

Strain	Growth Conditions	$K_{0.5}(CO_2)$	
		pH 5.1	pH 7.3
		μM	
Wild-type	Air-grown	3	1
Wild-type	High CO_2-grown	13	20
CIA-5	Air-grown	18	27
CIA-5	High CO_2-grown	21	28

Table IV. *Measurement of External Carbonic Anhydrase Levels in Wild-Type and CIA-5 Cells*

Carbonic anhydrase activity was measured on intact cells and the values shown represent the average of three or more determinations.

Strain	Growth Conditions	Carbonic Anhydrase Activity
		units mg Chl^{-1}
Wild-type	Air-grown	610 ± 180
Wild-type	High CO_2-grown	35 ± 12
CIA-5	Air-grown	44 ± 5
CIA-5	High CO_2-grown	24 ± 12

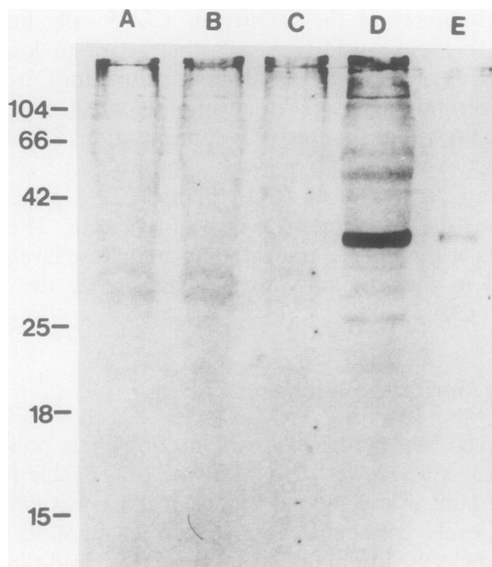

Figure 1. Immunoblot detection of the periplasmic carbonic anhydrase in *CIA-5* or wild-type cells. Cells were grown up on air supplemented with 5% CO_2 and then switched to either air or air supplemented with 5% CO_2 for 24 h prior to the experiment. Cell extracts equivalent to 10 μg of Chl were loaded in each lane. Lane A, high CO_2-grown *CIA-5* cells; lane B, air-grown *CIA-5* cells; lane C, high CO_2-grown wild type cells; lane D, air-grown wild type cells; lane E, isolated periplasmic carbonic anhydrase.

C_i Uptake Measurements

Air-grown *CIA-5* cells were tested for their ability to accumulate C_i, and were found to be unable significantly to concentrate C_i to levels higher than could be accounted for by diffusion (Fig. 2). Consistent with the $K_{0.5}(CO_2)$ measurements (Table III), low CO_2-grown *CIA-5* cells had very low C_i uptake characteristics, similar to the high CO_2-grown *CIA-5* cells. This is in contrast to the wild-type cells in which the air-grown cells have a much greater ability to accumulate C_i than the high CO_2-grown cells. Air-grown wild-type cells also accumulate C_i to much greater levels than *CIA-5* cells grown either on high or low CO_2 levels (Fig. 2). The C_i accumulation by *CIA-5* cells was always similar to that observed in wild-type high CO_2-grown cells, cells that did not have the CO_2 concentrating mechanism. In addition, the permeant carbonic anhydrase inhibitor, EZ (15), had little or no effect on C_i accumulation by *CIA-5* (Fig. 3). In wild-type, EZ caused an increased accumulation of C_i presumably by inhibiting an internal carbonic anhydrase (17, 21). In the experiments with EZ, the periplasmic carbonic anhydrase was also inhibited. If the periplasmic carbonic anhydrase was the only defect in *CIA-5* cells, the *CIA-5* cells would be expected to respond in a manner similar to wild-type cells. Instead the *CIA-5* cells consistently exhibit low C_i accumulation characteristics in the presence or absence of carbonic anhydrase inhibitors (Fig. 3).

These data indicated that *CIA-5* failed to adapt to low CO_2 conditions at all, and is defective not only in the periplasmic carbonic anhydrase. This idea was further supported by $K_{0.5}(CO_2)$ values determined at pH 5.1 (Table III). At this low pH, the contribution of the periplasmic carbonic anhydrase to the supply of C_i to the cell is minimal (16, 17). Therefore, if *CIA-5* was missing only the periplasmic carbonic anhydrase, the $K_{0.5}(CO_2)$ determinations at pH 5.1 should not be greatly different from the wild-type cells. However, this was not the case as the apparent affinity for CO_2 of air-grown *CIA-5* at pH 5.1 was much less than air-grown wild-type cells (Table III). These data implied that *CIA-5* may be missing other components of the C_i transport system in addition to the periplasmic carbonic anhydrase.

^{35}S-Labeling of *CIA-5*

Recently three polypeptides of 46 kD, 44 kD, and 20 kD, in addition to the 37 kD periplasmic carbonic anhydrase, have been implicated in the functioning of the CO_2 concentrating system in *C. reinhardtii* (13). These polypeptides were preferentially labeled when [^{35}S]sulfate was added to high CO_2-grown cells while adapting to low levels of CO_2 (13). When *CIA-5* cells were also labeled while adapting to low CO_2, none of the four polypeptides normally associated with

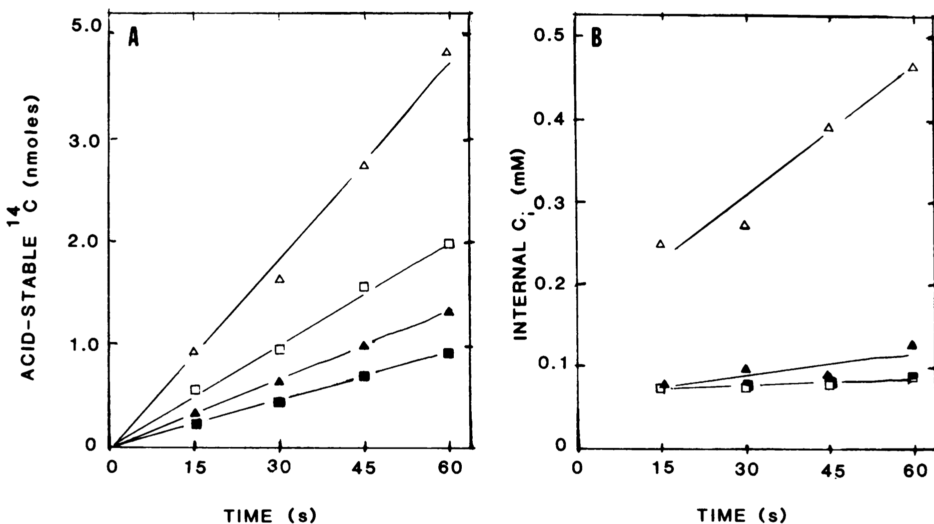

Figure 2. C_i accumulation and $^{14}CO_2$ fixation by *CIA-5* cells. $^{12}CO_2$ fixation (A) and C_i accumulation (B) were determined in wild-type (△, □) and *CIA-5* cells (▲, ○) that had been switched to air for 22 h (△, ▲), or air supplemented with 5% CO_2 (□, ■). C_i uptake was determined in 25 mM Hepes-KOH (pH 7.3) with the cell concentration at 25 μg Chl·ml^{-1}. Na-$H^{14}CO_3$ (40 μM) was added to CO_2-depleted cells at 0 time.

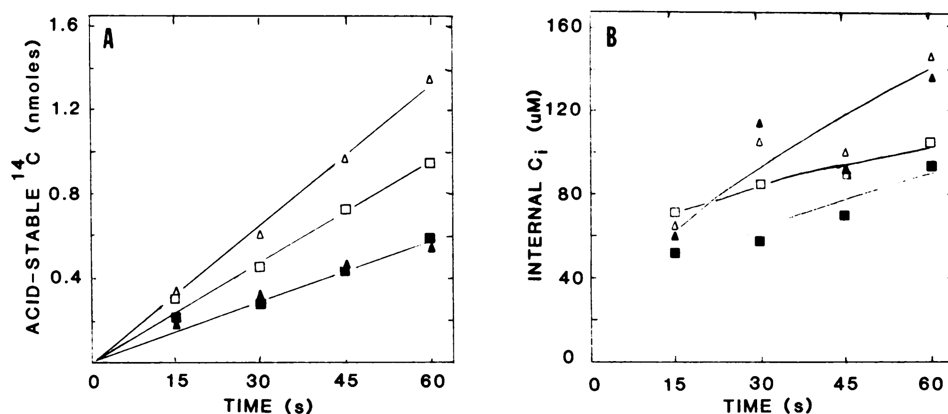

Figure 3. Effect of the carbonic anhydrase inhibitor, EZ, on inorganic carbon accumulation by *CIA-5* cells. $^{14}CO_2$ fixation (A) and C_i accumulation (B) were determined in *CIA-5* cells that had been switched to air for 20 h (△, ▲), or air supplemented with 5% CO_2 (□, ■). Cells were treated with 50 μM EZ (▲, ■), or tested in the absence of this inhibitor (△, □). C_i uptake was determined in 25 mM Hepes-KOH (pH 7.3) with the cell concentration at 25 μg Chl·ml^{-1}. $NaH^{14}CO_3^-$ (40 μM) was added to CO_2-depleted cells at 0 time.

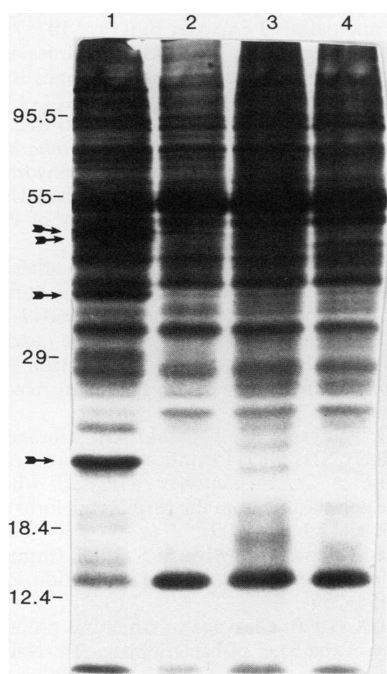

Figure 4. Autoradiogram of newly synthesized proteins made by wild-type or *CIA-5* cells grown with air or with elevated CO_2. Wild-type and *CIA-5* cells were grown on minimal media containing low sulfate and bubbled with air containing 5% CO_2 for 2 d prior to the experiment. Cells were harvested and then placed in fresh media containing no sulfate at a concentration of 25 mg Chl ml^{-1} and bubbled with air or air supplemented with 5% CO_2. After illuminating the cells for 1 h, $^{35}SO_4^{-2}$ was added and the cells were illuminated for an additional 3 h. These labeled cultures were then harvested, extracted with chloroform:methanol (1:1), and the precipitated proteins subjected to SDS-PAGE. Labeled proteins were then visualized by autoradiography. Lane 1, wild-type cells switched to low CO_2; lane 2, *CIA-5* cells switched to low CO_2; lane 3, wild-type cells kept on high CO_2; lane 4, *CIA-5* cells kept on high CO_2. Molecular weight markers are shown on the left. The arrows indicate polypeptides which were preferentially labeled by wild-type cells on low CO_2.

cells adapting to low CO_2 concentrations were observed (Fig. 4). These data support the contention that *CIA-5* is a *C. reinhardtii* mutant that is unable to adapt to low CO_2.

DISCUSSION

C. reinhardtii, like many other microalgae, can adapt to low CO_2 conditions by inducing a CO_2 concentrating mechanism (1–3). One protein that is important in the acquisition of CO_2 is the periplasmic carbonic anhydrase (6, 11, 31). This protein has a monomer molecular weight of 37 kD (31) and facilitates the diffusion of CO_2 into the algal cell. The other proteins proposed to be involved in inorganic carbon uptake have not been characterized. Manuel and Moroney (13) have identified four polypeptides that were synthesized by *C. reinhardtii* when high CO_2 grown cells were switched to low CO_2. One of these polypeptides was identified as the 37 kD periplasmic carbonic anhydrase. The role that the other three polypeptides play in C_i uptake is still unclear.

This manuscript describes a nuclear mutation of *C. reinhardtii* that requires high CO_2 for normal photoautotrophic growth. This strain is also unable to synthesize any of the four polypeptides associated with growth on low CO_2 in *C. reinhardtii* (13). A number of *C. reinhardtii* mutants deficient in some aspect of C_i acquisition have been isolated and partially characterized (18, 23–26). These mutants are unable to grow on low levels of CO_2 but are able to grow photoautotrophically on high levels of CO_2. The growth characteristics of *CIA-5* (Table I) are similar to the *C. reinhardtii* strains previously described which contain nuclear mutations that fall into three different complementation groups (18, 25, 26) and exhibit different C_i uptake characteristics. All of the mutants previously characterized contained normal or near-normal levels of the periplasmic carbonic anhydrase (18, 26). The mutation described in this report, *CIA-5*, is the first strain to be isolated that is missing the periplasmic carbonic anhydrase. This was demonstrated by the low levels of carbonic anhydrase activity measured in intact cells (Table IV) and the absence of the protein as determined by immunoblot analysis (Fig. 1). In addition to the periplasmic carbonic anhydrase (37 kD), the 45 and 110 kD polypeptides identified as possible intracellular forms of carbonic anhydrase (10) also appeared to be deficient in the *CIA-5* strain (Fig. 1).

In addition to lacking carbonic anhydrase, *CIA-5* was unable to accumulate C_i to levels normally seen in wild-type cells (Fig. 3). This was observed not only at pH 7.3 (Fig. 2) but also at pH 5.1 where most of the external C_i is in the form

of CO_2 and the physiological effects of the periplasmic carbonic anhydrase are minimal (16, 17). This was confirmed by the $K_{0.5}(CO_2)$ measurements at pH 5.1 (Table III). The *CIA-5* cells still had a low affinity for CO_2 despite the fact that inhibition of the periplasmic carbonic anhydrase with impermeant sulfonamides did not lower the affinity of wild-type cells for CO_2 at low pH (17). In addition, the permeant inhibitor, EZ, failed to cause an increase in C_i accumulation in *CIA-5* (Fig. 3). In air-grown, wild-type cells, this inhibitor caused an overaccumulation of C_i while inhibiting the periplasmic carbonic anhydrase and presumably an internal carbonic anhydrase (17). When *CIA-5* cells exposed to air levels of CO_2 are tested for C_i uptake in the presence of EZ, there was no increase in C_i accumulation (Fig. 3), implying some component(s) in addition to the periplasmic carbonic anhydrase were missing in *CIA-5*.

The C_i uptake studies and the $K_{0.5}(CO_2)$ measurements indicated that the *CIA-5* strain may be unable to adapt to low CO_2 at all. Previously characterized mutants have all been able to induce the synthesis of the periplasmic carbonic anhydrase when placed on low CO_2, indicating that they can partially adjust to the low CO_2 conditions. The idea that *CIA-5* is unable to adapt to low CO_2 was supported by the ^{35}S-labeling studies. In these experiments, none of the four polypeptides associated with the induction of the CO_2 concentrating mechanism were labeled in the *CIA-5* cells (Fig. 4). These experiments led further support to the hypothesis that the four polypeptides preferentially labeled under low CO_2 conditions are important in the functioning of the CO_2 concentrating mechanism. Previously, Manuel and Moroney (13) demonstrated that the *C. reinhardtii* mutant *pmp-1* failed to make two of these polypeptides with apparent molecular weights of 46 and the 44 kD but did make the 37 and the 20 kD polypeptides. This is consistent with the observation that *pmp-1* does induce the synthesis of the periplasmic carbonic anhydrase on exposure to low CO_2. In the case of the present mutant, all four of these polypeptides are missing. The absence of the 37 kD protein is consistent with the absence of detectable periplasmic carbonic anhydrase as judged by enzyme assay or by immunoblots.

These data, in addition to the C_i uptake data and the $K_{0.5}(CO_2)$ determinations, argue that the *CIA-5* strain is unable to induce the synthesis of several proteins neccessary to adapt to low CO_2 and may contain a mutation in the mechanism that controls the induction of the CO_2 concentrating system. This strain should provide a good control for researchers who want to compare *C. reinhardtii* cells that have the CO_2 concentrating mechanism to those that do not have it. In addition, this mutant may be valuable to researchers studying the induction of the CO_2 concentrating mechanism. Studies have been done on the physiological requirements for the induction of the CO_2 concentrating mechanism but very little is known about the molecular details of the control of this induction.

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