Plant Physiol. (1988) 88, 491–496
0032-0889/88/88/0491/06/$01.00/0

Inorganic Carbon Accumulation by *Chlamydomonas reinhardtii*[1]

NEW PROTEINS ARE MADE DURING ADAPTATION TO LOW CO_2

Received for publication February 21, 1988 and in revised form April 20, 1988

LIVINGSTON J. MANUEL AND JAMES V. MORONEY*
Department of Botany, Louisiana State University, Baton Rouge, Louisiana 70803

ABSTRACT

When the unicellular green alga *Chlamydomonas reinhardtii* is placed under low CO_2 conditions it adapts by making an inorganic carbon accumulating mechanism. Algal cells were labeled with $^{35}SO_4^{-2}$ during this adaptation period and labeled proteins specific for this low CO_2 adaptation were identified. Four major proteins were preferentially synthesized under low CO_2 conditions and had M_r of 46, 44, 37, and 20 kilodaltons. The 37 kilodalton protein is most likely the periplasmic carbonic anhydrase previously identified as being part of the inorganic carbon accumulation mechanism of *C. reinhardtii*. The other three proteins have not been identified. The 46 and the 44 kilodalton proteins were not synthesized by a mutant algal strain, *pmp-1*, which cannot grow at low CO_2 concentrations. This strain does make the 37 and 20 kilodalton proteins, however. These data suggest that at least two or three proteins in addition to the periplasmic carbonic anhydrase are part of the inorganic carbon accumulation mechanism in *C. reinhardtii*.

The unicellular green alga *Chlamydomonas reinhardtii* can grow photoautotrophically even when the external CO_2 concentration is very low (2). This ability to grow with low CO_2, lower than higher plants that use the C_3 pathway can tolerate, has been seen with a large number of algae across a wide phylogenic range (1, 5). Berry *et al.* (4) postulated that *C. reinhardtii* has the ability to accumulate inorganic carbon (C_i[2]) internally to much higher levels than could be obtained by diffusion. Uptake studies using gas exchange (26) or $^{14}CO_2$ and $H^{14}CO_3^-$ (2, 16, 17, 24, 25) have supported this hypothesis. The details of this C_i accumulation system are not well understood, however.

One component of the C_i accumulation mechanism of *C. reinhardtii* that has been identified is a carbonic anhydrase located in the periplasmic space (6, 27, 30, 31). This carbonic anhydrase is an oligomeric glycoprotein having a monomer M_r of 37 kD and is preferentially synthesized when *C. reinhardtii* is grown photoautotrophically with low levels of CO_2. When *C. reinhardtii* is placed under low CO_2 conditions the periplasmic carbonic anhydrase activity increases (8, 27) and the amount of this protein increases (6, 8, 31). This increase in carbonic anhydrase is likely to be under transcriptional control, as the levels of the mRNA coding for this protein also increase under low CO_2 conditions (8).

In this paper, we report on other proteins that are preferentially synthesized under low CO_2 conditions. This was done by radioactively labeling cells during their adaptation to low CO_2 and determining which proteins have increased synthesis. We present evidence that the proteins identified by this method may be part of the C_i accumulation mechanism of *C. reinhardtii*.

MATERIALS AND METHODS

Algal Strains and Culture Conditions. The wild-type strain of *Chlamydomonas reinhardtii*, 137 mt+, used in these experiments has been maintained in Dr. R. K. Togasaki's laboratory. The high CO_2-requiring strain, *pmp-1*, was a gift of Dr. M. H. Spalding and the arginine-requiring strain, CC1685, was obtained from the Duke University *Chlamydomonas* Culture Collection. Cultures were maintained on yeast-acetate plates until a few weeks before use. In liquid culture, the strains were routinely grown on minimal media (28) or in the case of CC1685, minimal media supplemented with 50 mg/L arginine. The liquid flasks were aerated with 5% CO_2 in air and illuminated with 300 $\mu E\ m^{-2}\ s^{-1}$ of white light. For $^{35}SO_4^{-2}$ labeling studies, wild-type or *pmp-l* cells were switched to minimal media with about one-fifth (140 *versus* 510 μM) the normal amount of sulfur between 24 and 48 h prior to the experiment. This media had one-tenth the normal $MgSO_4$ (40 *versus* 400 μM) and was supplemented with $MgCl_2$ to maintain the normal Mg^{2+} concentration present in minimal media. For experiments using [^{14}C]arginine the sulfur concentration was normal, but the CC1685 cells were kept in minimal media supplemented with arginine.

Labeling Cells with $^{35}SO_4^{-2}$. For these experiments, cells that had been growing on minimal media with one-fifth sulfur and aerated with 5% CO_2 were harvested by centrifugation at 3000 rpm in a JA-14 rotor for 5 min. The pellet was resuspended in minimal media lacking sulfate (Min-S) and centrifuged again. The pellet was again resuspended in Min-S media and the Chl concentration determined. The cells were then adjusted to 10 μg Chl/mL and divided into 100 mL flasks. These flasks were then bubbled with either air or air supplemented with 5% CO_2. To label the cultures, 5 to 10 μCi of carrier-free $^{35}SO_4^{-2}$ (1000 Ci/mmol) was added to the cultures at the times indicated in the figure legends.

After incubating the cells for the appropriate time with the label, the cells were harvested by centrifugation at 3,000 rpm for 5 min at 0 to 4°C. The cells were then washed twice with 50 ml of ice-cold 50 mM Tris-HCl (pH 7.4) and collected again by centrifugation. The cells were then extracted with 20 mL of chloroform:methanol (1:1) by mixing vigorously at room temperature. After mixing for 1 min, the extracted cells were pelleted by centrifugation at 5,000 rpm (JA-20 rotor) for 10 min. The green supernatant was discarded and the white precipitate resus-

[1] Supported by NSF Grant DMB-8703462, Louisiana Board of Regents Contract LEQSF (86-89)-RD-A-03 and a grant from the Louisiana State University Council on Research to J. V. M.

[2] Abbreviations: C_i, inorganic carbon; Min, minimal media; Min-S, minimal media containing $MgCl_2$ instead of $MgSO_4$ and chloride salts substituted for sulfates in the trace elements; Rubisco, ribulose-1,5-bisphosphate carboxylase-oxygenase; low CO_2, air containing ambient (340 ppm) CO_2 levels; high CO_2, air supplemented with CO_2 so that the final CO_2 concentration is 5% (v/v).

pended in 15 ml of 0.5% (w/v) TCA. The sample was then centrifuged at 5,000 rpm (JA-20 rotor) and the supernatant discarded. The pellet was then dried under N_2 and resuspended in between 0.5 and 1.5 ml of 0.083 M $NaCO_3$, 0.083 M DTT, 1.0% (w/v) SDS, and 5% sucrose, a buffer suitable for SDS polyacrylamide gel electrophoresis. To compare different treatments, samples were loaded to equal counts, usually between 50,000 and 100,000 CPM per lane.

Labeling of Cells with [^{14}C]Arginine. The procedures used here were essentially the same as described for the $^{35}SO_4^{-2}$ labeling except that the cells were resuspended in minimal media prior to the addition of [^{14}C]arginine (55 mCi/mmol) instead of $^{35}SO_4^{-2}$. The harvesting of the cells was the same as described in the last section.

Other Methods. SDS polyacrylamide gel electrophoresis was performed as previously described (13) with a 12% (w/v) acrylamide concentration unless otherwise noted. Autoradiography was performed using Kodak X-OMAT film. Chl was determined spectrophotometrically. The amounts of radioactivity incorporated into the algal cells was determined by taking aliquots of the extracted cells in buffer and counting the sample using a Beckman LS 1801 liquid scintillation counter.

Materials. [^{14}C]Arginine and $H_2{}^{35}SO_4$ were purchased from ICN.

RESULTS

Chlamydomonas reinhardtii, when grown photoautotrophically with elevated levels of CO_2, has a low affinity for C_i and has low levels of carbonic anhydrase. However, when a high CO_2-grown culture is switched to low CO_2 levels (air levels of CO_2) some of the alga's photosynthetic properties rapidly change. The cells' affinity for C_i increases and the levels of carbonic anhydrase dramatically increase (6, 8, 31). In addition, *C. reinhardtii* gains the ability to accumulate C_i to levels higher than can be obtained by diffusion (2, 16, 24). This adaptation to low CO_2 conditions is complete between 4 and 6 h after transferring the culture to air levels of CO_2 (6).

If cultures are labeled with $^{35}SO_4^{-2}$ during this adaptation to low CO_2, some proteins can be seen that are not labeled if the culture has remained growing at high CO_2 levels. Figure 1 is an autoradiogram of proteins labeled between 1 and 4 h after switching a culture to low CO_2 and of proteins labeled from a culture that had been left on high CO_2. Four proteins appear to be specifically labeled when *C. reinhardtii* cultures are switched to low CO_2. These proteins have apparent M_r of 46, 44, 37, and 20 kD (Fig. 1). The most likely identity of the 37 kD protein is the periplasmic carbonic anhydrase. This protein has a monomer mol wt of 37 kD and has been previously shown to be labeled under air adapting conditions similar to those described here (6, 8, 31). The identity of the other three proteins is unknown. In addition to these proteins whose synthesis appear to increase upon switching to low CO_2 conditions, the synthesis of other proteins appears to decrease. The most notable of these proteins are the large and small subunits of rubisco with M_r of 53 and 14 kD. The decrease in the synthesis of the rubisco subunits upon transfer to low CO_2 was previously seen by Coleman and Grossman (7). Other proteins which consistently have decreased synthesis on low CO_2 have apparent M_r of 38 and 25 kD.

If proteins from air-adapted cultures of *C. reinhardtii* are compared to those from high-CO_2 grown cells by Coomassie blue staining, there are few obvious differences (Fig. 2). No apparent staining differences can be seen in the regions where there are labeling differences between samples from air-adapted cultures and high-CO_2 grown cultures (Fig. 2). Since these are gels of crude homogenates, this only implies that none of these proteins are extremely abundant.

A time course of the labeling of these proteins is shown in

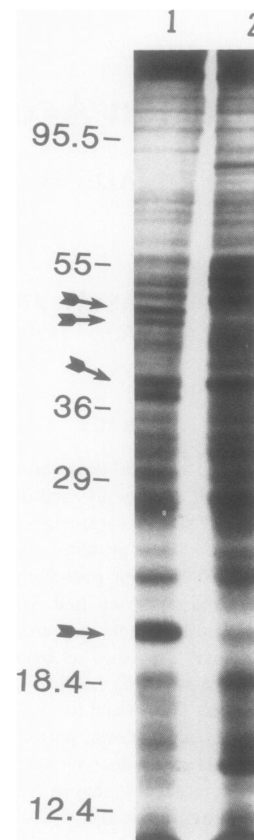

FIG. 1. Autoradiogram showing newly synthesized polypeptides from *C. reinhardtii* grown with 5% CO_2 or adapting to low CO_2. Five percent CO_2-grown cells were harvested as described in "Materials and Methods." One aliquot of the cells was bubbled with 5% CO_2 and one aliquot bubbled with air. The cells were bubbled for 1 h and then labeled with $^{35}SO_4^{-2}$ for 3 h while the bubbling continued. The cells were then harvested and subjected to SDS-polyacrylamide gel electrophoresis and autoradiography. Lane 1, air-adapting cells; lane 2, CO_2-grown cells. Mol wt markers as indicated to the left in the figure are phosphorylase B (95,500), glutamate dehydrogenase (55,000), ovalbumin (43,000), lactate dehydrogenase (36,000), bovine erythrocyte carbonic anhydrase (29,000), lactoglobulin (18,400), Cyt *c* (12,400).

Figure 3. Within 2 h after switching to low CO_2 from high CO_2 the rates of synthesis of all four proteins is maximal. The rates of synthesis of the 20, 37, and 46 kD proteins as exhibited by the incorporation of ^{35}S, then decreases as the cells adapt to low CO_2, but does not decrease to zero (Fig. 3). After 6 to 8 h the rates of synthesis of these three proteins remains at this lower level as long as the culture remains on low CO_2 (Fig. 3, lane 7; Fig. 4, lane 3). The 44 kD protein does not follow this pattern. The 44 kD protein appears shortly after the cells are switched to low CO_2 and synthesis of this protein apparently decreases dramatically after 4 h (Fig. 3). The low CO_2 requirement for the synthesis of these polypeptides was also seen when air-grown cultures were placed on high CO_2. When a culture growing on air levels of CO_2 is switched back to high CO_2, the labeling of these proteins ceases. The labeling of the 46, 44, and 20 kD protein stops within 3 h while the labeling of the 37 kD decreases more slowly (Fig. 4).

To get optimal labeling of newly made proteins, the *C. reinhardtii* cultures were first starved for sulfur by growing them on low S-containing media for 1 or 2 d. To determine whether the labeled proteins observed were really due to the low CO_2 conditions and not an artifact of the low sulfur growth, adapting cultures were labeled with ^{35}S in media containing normal levels

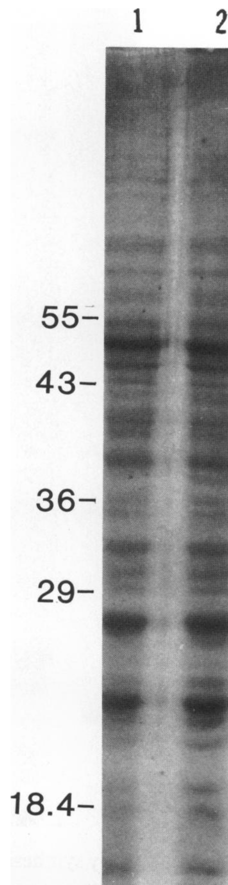

FIG. 2. Coomassie blue stained gels of proteins from air-grown or 5% CO_2-grown *C. reinhardtii* cells. Cells were harvested and subjected to SDS polyacrylamide gel electrophoresis as described in "Materials and Methods;" 20 μg of protein were loaded in each case. Lane 1, air-grown cells; lane 2, 5% CO_2-grown cells.

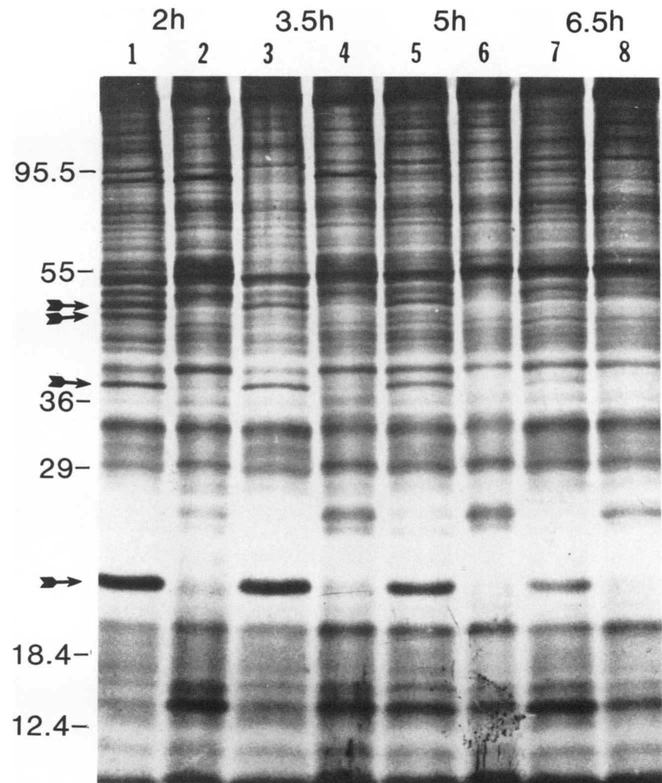

FIG. 3. Autoradiogram of time course showing the synthesis of polypeptides in *C. reinhardtii* after transfer of cells from high CO_2 levels to air. Lanes 1, 3, 5, and 7 were labeled while bubbling with air levels of CO_2. Lanes 2, 4, 6, and 8 were labeled while bubbling with air supplemented with 5% CO_2. The samples in lanes 1 and 2 were labeled from 0 time to 2 h, those in lanes 3 and 4 from 1.5 to 3.5 h, those in lanes 5 and 6 from 3 to 5 h, and those in lanes 7 and 8 from 4.5 to 6.5 h. Cells were labeled with $^{35}SO_4^{-2}$ during growth in minimal-S media. The cultures were harvested as described in text and the fractions obtained, subjected to SDS-PAGE and autoradiography. Numbers to the left indicate the position of the mol wt markers as described in the legend to Figure 1.

of sulfate. When these cultures were placed on low CO_2 and subsequently labeled with $^{35}SO_4^{-2}$, the same preferentially labeled proteins are observed (Fig. 5). While the overall labeling pattern is somewhat different from the low ^{35}S labeling experiment, the same proteins are specifically labeled only in the low-CO_2 grown cells using $^{35}SO_4^{-2}$ when the sulfate concentration is high or low. This experiment argues that these proteins are not associated with the low sulfur growth conditions but are likely to be proteins specifically made under low CO_2. In addition, an arginine-requiring strain CC1685 was labeled with [^{14}C]arginine under both high and low CO_2 conditions in media containing normal sulfate levels. The 44, 37, and 20 kD were labeled more strongly under air conditions. The 46 kD protein, however, was obscured by a strongly labeled protein that also migrated at this position (data not shown). This experiment further supports the argument that the 44, 37, and 20 kD polypeptides are not related to sulfur metabolism but does not argue for or against the 46 kD polypeptide.

The mutant cell line *pmp-1* was also labeled while adapting to low CO_2. This mutant cannot grow under low CO_2 and cannot accumulate C_i (25, 26). However, *pmp-1* does synthesize the periplasmic carbonic anhydrase when placed at low CO_2 (18, 26). When *pmp-1* cells are labeled while adapting to low CO_2, the 37 kD and the 20 kD proteins are labeled but the 46 and the 44 kD proteins are absent (Fig. 6). These results suggest that the inability of the *pmp-1* cells to accumulate C_i and adapt to low CO_2 conditions may be due to their inability to synthesize the 46 and the 44 kD proteins, and that these proteins may be important to the functioning of the C_i accumulation mechanism.

DISCUSSION

Chlamydomonas reinhardtii, when grown with high levels of CO_2, exhibits many of the same photosynthetic gas exchange characteristics of C_3 plants, including a low affinity for CO_2 and high rates of photorespiration (2, 16, 20, 25, 26). However, *C. reinhardtii*, like many other unicellular algae, can also adapt to low CO_2 conditions (1, 5, 15). When grown with low levels of CO_2 the same as those in ambient air, the alga exhibits a high affinity for CO_2 and apparently low rates of photorespiration (20). Researchers have hypothesized that this ability to efficiently use CO_2 is due to the ability of these air adapted *C. reinhardtii* cells to accumulate C_i internally (2); however, the mechanism of this C_i accumulation is not clear.

Previously, the only protein identified with the C_i accumulation mechanism in *C. reinhardtii* was carbonic anhydrase. In the case of *C. reinhardtii*, a 37 kD carbonic anhydrase is made when cells are placed under low CO_2 conditions (6, 8, 31). This protein is located in the periplasmic space of *C. reinhardtii* (11, 27, 30). There is considerable evidence, however, that this carbonic anhydrase is not the only protein associated with the ability of *C. reinhardtii* to accumulate C_i. Perhaps the strongest evidence

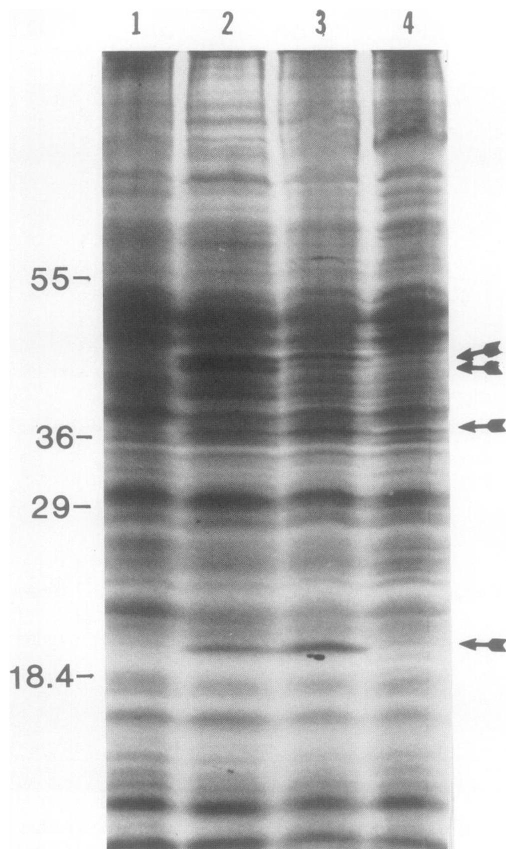

FIG. 4. Autoradiograms of newly synthesized polypeptides from air grown cells and air grown cells placed on high levels of CO_2. A *C. reinhardtii* culture maintained on 5% CO_2 was divided into two flasks containing low S (140 μM SO_4^{-2}) minimal media. One was maintained on high CO_2 and one placed on air. These cultures were grown in the light on these gas regimes for 26 h prior to the experiment. At that time both cultures were harvested as described in "Materials and Methods" and both were split into two parts, one bubbled with air and the other bubbled with air supplemented with 5% CO_2. These cultures were labeled with ^{35}S 1 h after harvest and labeled for a 2 h period. Lane 1, 5% CO_2-grown culture maintained on 5% CO_2; lane 2, 5% CO_2-grown culture switched to air levels of CO_2; lane 3, air-grown culture maintained on air; lane 4, an air-grown culture switched on 5% CO_2. The mol wt markers to the left of the figure are the same used in Figure 1.

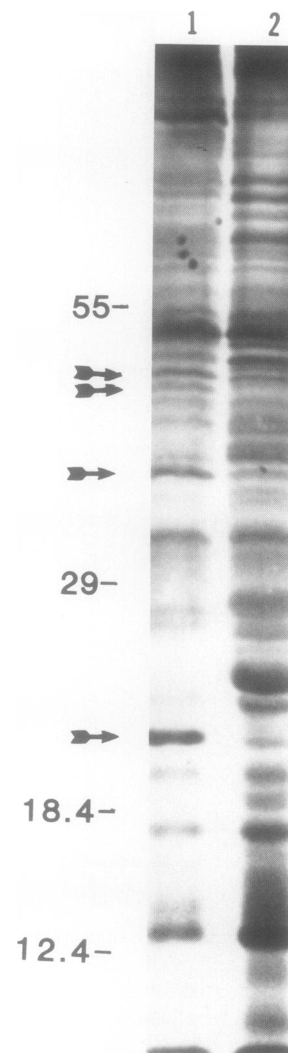

FIG. 5. Autoradiogram of *C. reinhardtii* showing polypeptide synthesis on normal sulfur levels. Cells were grown in minimal media and bubbled with 5% CO_2. Cells were then harvested, and one aliquot illuminated in the presence of air and another illuminated in the presence of air with 5% CO_2. The cultures were incubated for 1 h in minimum media prior to the addition of label. After including $^{35}SO_4^{-2}$ the cultures were labeled for 3 h. Harvesting of cultures was as described in "Materials and Methods." The fractions obtained were subjected to SDS-PAGE and autoradiography. Lane 1, cells switched to air; lane 2, cells on 5% CO_2. Numbers to the left indicate the positions of the mol wt markers which were described in the legend to Figure 1.

comes from the identification of mutants of *C. reinhardtii* that cannot grow on low levels of CO_2 but can grow photoautotrophically with elevated CO_2 concentrations (18, 24–26). These mutants, of which *pmp-1* is one example, (26) have aberrant C_i accumulation characteristics but make normal amounts of the external carbonic anhydrase. These mutants indicate that other proteins are necessary for a functional C_i accumulation mechanism.

The *in vivo* labeling data presented here further support the hypothesis that proteins other than the external carbonic anhydrase are involved in C_i accumulation of *C. reinhardtii*. The proteins most strongly labeled under low CO_2 growth conditions have apparent M_r of 46, 44, 37, and 20 kD. These polypeptides were also noted by Coleman and Grossman (7) when they placed *C. reinhardtii* on low CO_2. These proteins are labeled specifically when CO_2 conditions are low and $^{35}SO_4^{-2}$ is added to the cells. Low sulfur conditions can induce the synthesis of proteins not involved in carbon metabolism particularly those required for sulfur uptake in *C. reinhardtii* (9), but it is unlikely that the four proteins seen under our growth conditions are related to sulfur metabolism for three reasons. (a) The first reason is that we never see these four labeled proteins in the high-CO_2 control cells. These high-CO_2 grown cells have had the same low sulfur growth conditions as the cells placed on low CO_2. (b) The second reason is that the proteins of sulfur metabolism made under low sulfur conditions have different M_r than the proteins reported here. For example, de Hostos *et al.* (9) have identified a 72 kD periplasmic arylsulfatase that is synthesized by *C. reinhardtii* under low sulfur conditions. The polypeptides we see do not correspond to proteins known to be preferentially synthesized by *C. reinhardtii* under low sulfur conditions (9). (c) Finally, the labeling studies done at high sulfate conditions using either $^{35}SO_4^{-2}$ or [^{14}C] arginine would argue that these proteins are unrelated to sulfur transport and metabolism. The sulfur content of the growth media was maintained at the normal level during these experi-

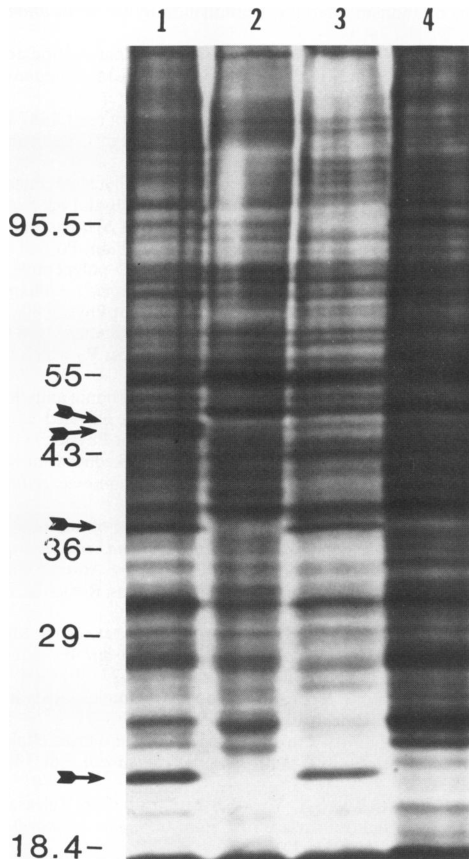

FIG. 6. Autoradiogram showing polypeptide synthesis in wild type *C. reinhardtii* and the high CO_2 requiring mutant *pmp-1*. Wild type and *pmp-1* cultures were grown on low S minimal media and bubbled with air supplemented with 5% CO_2. Prior to being labeled with $^{35}SO_4^{-2}$ the cultures were bubbled for 1 h. After labeling, the cultures were incubated for 3 h and harvested as described in the text. Fractions obtained were subjected to SDS polyacrylamide gel electrophoresis on a 10% gel. Mol wt markers as indicated to the left in the figure are described in the legend to Figure 1. Lane 1, wild-type cells switched to air; lane 2, wild-type cells on high CO_2; lane 3, *pmp-1* cells switched to air; lane 4, *pmp-1* cells on high CO_2.

ments. A possible exception in the arginine labeling experiment was the 20 kD protein. This protein was not as strongly labeled when [^{14}C]arginine was used although it did label more strongly when the cells were placed on air. It may have a high percentage of sulfur containing amino acids or may be regulated by the combination of carbon and sulfur availability. The 20 kD protein also showed the most variability during the ^{35}S experiments. It was always labeled under low CO_2 condition and never on high CO_2 but the intensity of labeling varied between experiments (see Fig. 1 *versus* Fig. 4).

The protein having a M_r of 37 kD is likely to be the periplasmic carbonic anhydrase. This protein has already been shown to be preferentially synthesized when the CO_2 concentration is low (6, 8) and has a monomer M_r of 37 kD. In addition, antibodies raised against the periplasmic carbonic anhydrase will immunoprecipitate a protein having the same M_r as the labeled band at 37 kD reported here (31).

The functions of the 46, 44, and 20 kD proteins are not known at present. The 44 kD polypeptide may be related to the periplasmic carbonic anhydrase since this carbonic anhydrase has been reported to be made as a precursor of either 42 (8) or 44 kD (29). We do not consider this to be a likely possibility because the 44 kD polypeptide identified here is more heavily labeled than the 37 kD protein even during 2 to 3 h labeling experiments. Toguri *et al.* (29) demonstrated that the labeled precursor chased into the mature protein within 10 min. In addition, the *pmp-1* strain failed to make significant amounts of either the 44 or the 46 kD polypeptides but does make the 37 kD protein and has normal amounts of the periplasmic carbonic anhydrase.

The only other protein associated with a C_i accumulation mechanism that has been identified is a 42 kD protein reported in *Anacystis nidulans* (22). This protein has been located on the cytoplasmic membrane of this cyanobacteria and appears to be related to HCO_3^- transport. In addition Ogawa *et al.* (21) and Omata *et al.* (23) have reported on mutants of *A. nidulans* that cannot grow in low CO_2 concentrations and have altered polypeptide compositions of their cytoplasmic membranes. In *C. reinhardtii*, the *pmp-1* mutant apparently cannot synthesize the 46 and 44 kD proteins (Fig. 6). This mutant originally described by Spalding *et al.* (25) is unable to grow under low CO_2 conditions and does not accumulate C_i to the high levels observed in wild-type cells (25, 26). Spalding *et al.* (25) speculated that the *pmp-1* mutant was defective in a C_i transport protein leading to the inability of this strain to accumulate C_i. While we have no evidence that identifies the function of the 46 and 44 kD proteins, the fact that these two polypeptides are missing in the *pmp-1* mutant supports the contention that these proteins are involved in the C_i accumulation mechanism.

While a number of investigators agree that a C_i transport protein must be involved in some way in the accumulation mechanism (14, 19, 26), the location of this transport protein and the C_i species transported is debated (14, 19). Two possible locations for the transport protein include the plasma membrane (14) and the chloroplast envelope (3, 19). In addition, both CO_2 and a HCO_3^- may be the carbon species transported. In cyanobacteria, Kaplan (10) has proposed a carbonic anhydrase-like transport protein. We are presently determining if the labeled proteins reported here are membrane bound and, if so, to which membrane they are associated.

Finally, it is possible that some of these labeled proteins are carbonic anhydrase isozymes, or are at least similar to carbonic anhydrase. Besides the carbonic anhydrase-like transport protein proposed by Kaplan, other models propose a chloroplastidic carbonic anhydrase (15 19) and possibly one located in the cytoplasm (17) in addition to the one located in the periplasmic space. Kitayama *et al.* (12) recently identified some proteins that were weakly immunoreactive with antibodies made to spinach chloroplast carbonic anhydrase. Two of these polypeptides have M_r close to 45 kD. Further experiments will be done to see if this immunoreactive protein is the same as one of the labeled proteins reported here.

In summary, we conclude that at least two, and possibly three proteins, in addition to the periplasmic carbonic anhydrase are important for the C_i accumulation mechanism in *C. reinhardtii*. Since these labeling studies were done to crude cell homogenates it is very possible that additional proteins will be identified in the future. With the possible exception of the 20 kD protein which was somewhat variable in our hands, these are not particularly abundant proteins, but they are strongly labeled when *C. reinhardtii* cells are transferred to low CO_2. In addition, two of these proteins are not made by the high-CO_2-requiring mutant *pmp-1*, pointing to a possible physiological role for these proteins.

Acknowledgments—The authors thank Dr. Sue Bartlett for many helpful suggestions and Catherine B. Mason for excellent technical assistance. We also thank Dr. Russell Chapman and Dr. Bartlett for sharing their space and equipment during the initial phase of these experiments.

LITERATURE CITED

1. AIZAWA K, S MIYACHI 1986 Carbonic anhydrase and CO_2 concentrating mechanisms in microalgae and cyanobacteria. FEMS Microbiol Rev 39: 215–233

2. BADGER MR, A KAPLAN, JA BERRY 1980 Internal inorganic carbon pool of *Chalamydomonas reinhardtii*: evidence for a carbon dioxide concentrating mechanism. Plant Physiol 66: 407–413
3. BEARDALL J 1981 CO_2 accumulation by *Chlorella saccharophila* (Chlorophyceae) at low external pH: evidence for the active transport of inorganic carbon at the chloroplast envelope. J Phycol 17: 371–373
4. BERRY JA, J BOYNTON, A KAPLAN, MR BADGER 1976 Growth and photosynthesis of *Chlamydomonas reinhardtii* as a function of CO_2 concentration. Carnegie Inst Wash Year Book 75: 423–432
5. BURNS BD, J BEARDALL 1987 Utilization of inorganic carbon by marine microalgae. J. Exp Mar Biol Ecol 107: 75–86
6. COLEMAN JR, JA BERRY, RK TOGASAKI, AR GROSSMAN 1984 Identification of extracellular carbonic anhydrase of *Chlamydomonas reinhardtii.* Plant Physiol 76: 472–477
7. COLEMAN JR, AR GROSSMAN 1983 Regulation of protein synthesis during adaptation of *Chlamydomonas reinhardtii* to low CO_2. Carnegie Inst Wash Year Book 82: 109–112
8. COLEMAN JR, AR GROSSMAN 1984 Biosynthesis of carbonic anhydrase in *Chlamydomonas reinhardtii* during adaptation to low CO_2. Proc Natl Acad Sci USA 81: 6049–6053
9. DE HOSTOS EL, RK TOGASAKI, AR GROSSMAN 1988 Purification and biosynthesis of a derepressible, periplasmic arylsulfatase from *Chlamydomonas reinhardtii.* J Cell Biol 106: 29–37
10. KAPLAN A 1985 Adaptation to CO_2 levels: induction and mechanism for inorganic carbon uptake. *In* WS Lucas, JA Berry, eds, Inorganic Carbon Uptake by Aquatic Organisms. American Society of Plant Physiologists, Rockville, MD, pp 325–338
11. KIMPEL DL, RK TOGASAKI, S MIYACHI 1983 Carbonic anhydrase in *Chlamydomonas reinhardtii.* I. Localization. Plant Cell Physiol 24: 255–259
12. KITAYAMA M, RK TOGASAKI, HD HUSIC 1987 Immunological analysis of carbonic anhydrase in *Chlamydomonas reinhardtii.* Plant Physiol Suppl 83: S-70
13. LAEMMLI UK 1970 Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227: 680–685
14. MARCUS Y, M VOLOKITA, A KAPLAN 1984 The location of the transporting system for inorganic carbon and the nature of the form translocated in *Chlamydomonas reinhardtii.* J Exp Bot 35: 1136–1144
15. MIYACHI S, M TSUZUKI, ST AVRAMOVA 1983 Utilization modes of inorganic carbon for photosynthesis in various species of *Chlorella.* Plant Cell Physiol 24; 441–451
16. MORONEY JV, NE TOLBERT 1985 Inorganic carbon uptake by *Chlamydomonas reinhardtii.* Plant Physiol 77: 253–258
17. MORONEY JV, HD HUSIC, NE TOLBERT 1985 Effect of carbonic anhydrase inhibitors on inorganic carbon accumulation by *Chlamydomonas reinhardtii.* Plant Physiol 79: 177–183
18. MORONEY JV, NE TOLBERT, BB SEARS 1986 Complementation analysis of the inorganic carbon concentrating mechanism of *Chlamydomonas reinhardtii.* Mol Gen Genet 204: 199–203
19. MORONEY JV, M KITAYAMA, RK TOGASAKI, NE TOLBERT 1987 Evidence for inorganic carbon transport by intact chloroplasts of *Chlamydomonas reinhardtii.* Plant Physiol 83: 460–463
20. MORONEY JV, BJ WILSON, NE TOLBERT 1987 Glycolate metabolism and excretion by *Chlamydomonas reinhardtii.* Plant Physiol 82: 821–826
21. OGAWA T, T KANEDA, T OMATA 1987 A mutant of *Synechococcus PCC7942* incapable of adapting to low CO_2 concentration. Plant Physiol 84: 711–715
22. OMATA T, T OGAWA 1986 Biosynthesis of a 42-kD polypeptide in the cytoplasmic membrane of the cyanobacterium *Anacystis nidulans* strain R_2 during adaptation to low CO_2 concentration. Plant Physiol 80: 525–530
23. OMATA T, T OGAWA, Y MARCUS, D FRIEDBERG, A KAPLAN 1987 Adaptation to low CO_2 level in a mutant of *Anacystis nidulans* R_2 which requires high CO_2 for growth. Plant Physiol 83: 892–894
24. SPALDING MH, RJ SPREITZER, WL OGREN 1983 Carbonic anhydrase deficient mutant of *Chlamydomonas reinhardtii* requires elevated carbon dioxide concentrations for photoautotrophic growth. Plant Physiol 73: 268–272
25. SPALDING MH, RJ SPREITZER, WL OGREN 1983 Reduced inorganic carbon transport in a CO_2-requiring mutant of *Chlamydomonas reinhardtii.* Plant Physiol 73: 273–276
26. SPALDING MH, RJ SPREITZER, WL OGREN 1985 Use of mutants in analysis of the CO_2-concentrating pathway of *Chlamydomonas reinhardtii. In* WJ Lucas, JA Berry, eds, Inorganic Carbon Uptake by Aquatic Photosynthetic Organisms. American Society of Plant Physiologists, Rockville, MD, pp 361–375
27. SPENCER KG, DL KIMPEL, ML FISHER, RK TOGASAKI, S MIYACHI 1983 Carbonic anhydrase in *Chlamydomonas reinhardtii* II: requirements for carbonic anhydrase induction. Plant Cell Physiol 24: 301–304
28. SUEOKA N 1960 Mitotic replication of deoxyribonucleic acids in *Chlamydomonas reinhardtii.* Proc. Natl Acad Sci USA 46: 83–91
29. TOGURI T, S MUTO, S MIYACHI 1986 Biosynthesis and intracellular processing of carbonic anhydrase in *Chlamydomonas reinhardtii.* Eur J Biochem 158: 443–450
30. YAGAWA Y, K AIZAWA, S-Y YANG, S MIYACHI 1986 Release of carbonic anhydrase from the cell surface of *Chlamydomonas reinhardtii* by trypsin. Plant Cell Physiol 27: 215–221
31. YANG S-Y, M TSUZUKI, S MIYACHI 1985 Carbonic anhydrase of *Chlamydomonas*: purification and studies on its induction using antiserum against *Chlamydomonas* carbonic anhydrase. Plant Cell Physiol 26: 25–34