Photosynthetic Carbon Metabolism in Photoautotrophic Cell Suspension Cultures Grown at Low and High CO₂

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ABSTRACT

Photosynthetic carbon metabolism was characterized in four photoautotrophic cell suspension cultures. There was no apparent difference between two soybean (Glycine max) and one cotton (Gossypium hirsutum) cell line which required 5% CO₂ for growth, and a unique cotton cell line that grows at ambient CO₂ (660 microliters per liter). Photosynthetic characteristics in all four lines were more like C₃ mesophyll leaf cells than the cell suspension cultures previously studied. The pattern of ^14C-labeling reflected the high ratio of ribulosebisphosphate carboxylase to phosphoenolpyruvate carboxylase activity and showed that CO₂ fixation occurred primarily by the C₃ pathway. Photosynthesis occurred at 330 microliters per liter CO₂, 21% O₂ as indicated by the synthesis of high levels of ^14C-labeled glycine and serine in a pulse-chase experiment and by oxygen inhibition of CO₂ fixation. Short-term CO₂ fixation in the presence and absence of carbonic anhydrase showed CO₂, not HCO₃⁻, to be the main source of inorganic carbon taken up by the low CO₂ requiring cotton cells. The cells did not have a CO₂-concentrating mechanism as indicated by silicone oil centrifugation experiments. Carbonic anhydrase was absent in the low CO₂-requiring cotton cells, present in the high CO₂-requiring soybean cell lines, and absent in other high CO₂ cell lines examined. Thus, the presence of carbonic anhydrase is not an essential requirement for photoautotrophy in cell suspension cultures which grow at either high or low CO₂ concentrations.

MATERIALS AND METHODS

Photoautotrophic Cell Suspension Cultures

Soybean (Glycine max) cells and high CO₂-requiring cotton (Gossypium hirsutum) cells were grown at 5% CO₂ in a modified MS medium which contained thiamine and hormones as the only organic compounds (22). Cells were subcultured every 2 weeks. Low CO₂-requiring cotton cells were grown at ambient CO₂, which was 660 μL/L, in a modified MS medium (3, 10), and were subcultured every 4 weeks. Cells were used in experiments 1 to 2 weeks after transfer, and were regularly found to be 90 to 95% viable as determined by phenosafranine staining (30). Prior to use, the cells were washed three times with the appropriate buffer (usually 30 mM Mops, pH 7.0). Cells stored for 4 h in buffer at 11 μE/m²·s in a test tube or Petri dish showed less than a 10% decrease in the rate of ^14CO₂-fixation.

CO₂ Fixation

Cells (10–20 μg Chl) in 1 mL buffer containing at least 200 IU carbonic anhydrase in sealed glass vials were placed on a shaker in a 30°C water bath. They were illuminated from below (300 μE/m²·s) and flushed with the desired CO₂, O₂, N₂ mixtures for 5 min. ^14CO₂ fixation was initiated by the addition of a trace amount of [^14C]NaHCO₃ (0.5 μCi, 54 Ci/mol). Reactions were quenched with 0.3 to 0.5 mL 3 N formic acid in methanol. Samples were dried at 65°C and resuspended in 0.2 mL HCl. Dpm were measured by liquid scintillation spectrometry. For experiments at saturating CO₂, reactions were initiated with 210 mM [^14C]NaHCO₃ (0.5 Ci/mol), bringing the final concentration to 10 mM NaHCO₃. Reaction rates were linear with time for at least 10 min and proportional to Chl up to at least 30 μg/mL. In the pulse-chase experiment the vials were flushed with 335 μL/L CO₂, 21% O₂, balance N₂ at 2 L/min after 2 min of ^14C-fixation. Control vials without cells showed that 90% of the ^14CO₂ was removed in 3 min. Vials were placed in a clear waterbath and illuminated from the side with a slide projector. CO₂ concentrations were calculated based on a PKa of 6.3 for HCO₃⁻/CO₂.

ABBREVIATIONS: MS medium, Murashige-Skoog medium; Caps, 3-[clohexylamino]-1-propanesulfonic acid; PEP, phosphoenolpyruvate; RuBP, ribulose-1,5-bisphosphate.

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14C-Label Distribution

Dried samples were applied twice with 500 μL H2O. The filtered solution was applied to 0.5 x 3.5 cm columns of Dowex 50W-X8 (100–200 mesh, H+ form) and eluted with 2 mL H2O, then 5 mL 2 N NH4OH, [14C]Gly, -Gln, and -Glu eluted in the NH4OH (B) fraction. The neutral fraction from Dowex 50 was applied to the same size Dowex 1-X8 column (100–200 mesh, formate form) and eluted with 3 mL H2O, 10 mL 4 N formic acid, 10 mL 10 N formic acid, and 10 mL 4 N HCl. These fractions were designated N, A1, A2, and A3, respectively. [14C]Sorbitol eluted in the N fraction, malate and glucose-1-P in A1, 3P-glycerate in A2, and fructose-1,6-P2 in A3. Recovery was at least 95%. No additional label eluted in 1 M HCl. Carboxylic acids in the A1 fraction, where indicated, were separated on an HPLC column (Bio-Rad HPX-87H) at room temperature with 4 mM H2SO4. Malate was identified by coelution with an authentic sample and by conversion to pyruvate with malic enzyme in 20 mM Hepes (pH 7.2), 1 mM NADP, 0.5 mM MnCl2. Fraction B was further purified in some cases (e.g., two-dimensional TLC on Analtech MN300 Cellulose plates [20 x 20 cm]) without fluorescent indicator, and autoradiographed (7). Samples were scraped, extracted, and counted (1) except that no Protosol was added. Recovery of 14C applied to the TLC plates was 95 to 100%. Amino acids were identified by comparison with ninhydrin-developed standards. Incubation of a sample with alanine dehydrogenase in 50 mM Caps (pH 10), 1 mM NAD, and 0.1 mM alanine resulted in the complete loss of [14C]alanine. Insoluble material was digested with H2O2 and HClO4 (14) or with α-amylase in 0.1 M KOAc (pH 4.8), 55°C, 20 h.

CO2 Response Curve

Reactions were conducted in specially crafted glass vials in order to ensure rapid equilibration of CO2 between the aqueous and gaseous phases. The vials were approximately 1.8 cm wide and 0.8 cm high with an 0.8 cm tapered neck to fit septa. Vials containing cells (10 μg Chl) in 1 mL 30 mM Mops (pH 7.0) and 200 IU carbonic anhydrase were illuminated and flushed with 21% O2 for 10 min. Reactions were initiated by the addition of [14C]NaHCO3 and were quenched after 1.0 min. Solutions were transferred to scintillation vials, dried, and counted as above.

Dark Respiration

Dark respiration was measured with an IR gas analyzer in a temperature-controlled, humidified system (26). Cells (50–300 μg Chl) were suspended in 30 mM Mops (pH 7.0), and at least 200 IU/mL carbonic anhydrase in a small open Petri dish to ensure rapid exchange of CO2 between the gaseous and aqueous phases. Dark respiration rate was proportional to Chl concentration.1

1 Mention of a trademark, proprietary product, or vendor does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture and does not imply its approval to the exclusion of other products or vendors that may also be suitable.

RuBP Carboxylase Assays

Cells were illuminated at 350 μE/m2·s unless otherwise indicated for 5 min in a Petri dish containing 30 mM Mops (pH 7.0), and at least 200 IU/mL carbonic anhydrase. The suspensions were swirled to prevent settling and were poured into an illuminated mortar prefrozen with liquid N2 containing frozen homogenization buffer. The final solution contained approximately 50 mM Bicine (pH 8.0) 10 mM MgCl2, 1 mM EDTA, 5 mM DTT, 0.1 mM leupeptin, 0.1 mM antipain, and 1 mM PMSF. Examination under a microscope showed that the cells were completely homogenized. An aliquot was removed for later Chl determination, and the homogenate was centrifuged for 3 s in a microfuge at 4°C. Initial activity was measured immediately by the addition of an aliquot of clarified homogenate to the reaction solution. Total activity was measured after incubating the clarified homogenate for 3 to 5 min at 30°C in a 1:1 dilution with 0.2 mM 6-P-gluconate, 50 mM Bicine (pH 8.0), 40 mM MgCl2, 40 mM NaHCO3. A time course showed that activation was complete. The final reaction solution contained 50 mM Bicine (pH 8.0), 10 mM MgCl2, 10 mM [14C]NaHCO3, and 0.4 mM RuBP in a sealed scintillation vial. Reactions were quenched after 30 s with 0.25 M 3 N formic acid in methanol. Reactions were linear up to 60 s. Controls with heated cell extract or minus RuBP showed no reaction. Samples were dried and counted as described above.

PEP Carboxylase Assays

Cell homogenate was prepared as described above. Enzyme activity was determined spectrophotometrically at 340 nm in 2.5 mM PEP, 10 mM NaHCO3, 5 mM MgCl2, 1 mM glucose-6-P, 1 mM DTT, 0.1 mM EDTA, 0.2 mM NADH, and 50 mM Bicine (pH 8.0) and 5 IU/mL malate dehydrogenase. A rapid initial decrease in absorbance was observed upon the addition of extract which was allowed to stabilize before the reaction was initiated with PEP. The rates were calculated after subtraction of the background reaction observed with heated samples. Reactions were conducted in the presence and absence of 16 IU/mL lactate dehydrogenase. Reactions without NaHCO3 were conducted in sealed cuvettes using solutions that had been degassed on ice for 4 h. Addition of PEP and PEP carboxylase showed a small amount of HCO3 to be present.

Inorganic Carbon

Cellular inorganic carbon concentrations in cells were determined following centrifugation through a 1:2 mixture of SF 96–50 and Versilube F50 (General Electric) silicone oils into 1 mL glycerine (pH 10) and 0.75% SDS (2). Photosynthesis was measured in 30 mM Mops (pH 7.0) and at least 200 IU/mL carbonic anhydrase. Continuous mixing was required for constant rates of CO2 fixation. Cell volume was determined using 3H2O and [14C]sorbitol (9).

Carbonic Anhydrase

Cells or leaf material were frozen in liquid N2 and homogenized in 25 mM Barbital (pH 8.3) and 5 mM DTT. Assays
(27) were conducted with several aliquots of extract containing different amounts of Chl because, when greater than 50 μg Chl was present, the calculated activity (IU/mg Chl) decreased.

Immunoblotting

Frozen leaf material and cells were homogenized in 30 mM Tris (pH 9.0), 1 mM PMSF, 14 mM β-mercaptoethanol and were clarified by centrifugation. Prior to homogenization of cotton leaves, 1% SDS was added to prevent protein precipitation. Rabbit polyclonal antibodies to spinach carboxic anhydrase (obtained from C. R. Somerville, Michigan State University) were used to probe nitrocellulose blots of 12.5% SDS-PAGE gels. The spots were developed with goat anti-rabbit IgG conjugated to alkaline phosphatase using indolylphosphate and nitroblue tetrazolium as described in the Protoblot System bulletin (Promega Biotech).

Chl and Protein

Chl was determined after extraction with ethanol (31). Protein concentration was determined with the Bio-Rad protein dye-reagent and crystalline BSA as the standard (4).

RESULTS

Characterization

Cotton cells grown at the culture room ambient CO2 concentration (660 μL/L) (COT-PA) were physically identical in size and shape to those grown at 5% CO2 (COT-P), except that the packed cell volume of the COT-PA line was as high as 6 to 8 mL/mg Chl compared to 2 to 3 mL/mg Chl for the COT-P line. Observations with a light microscope indicated that the cells were 35 to 60 μm in diameter and were mostly spherical, with some cells elongated up to 120 μm. Cells were largely vacuolar with chloroplasts studding the periphery. Extracts of both cotton cell lines contained 66 μg Chl/mg protein, compared with 63 and 47 μg Chl/mg protein in the 5% CO2-requiring soybean cell suspensions SB-P and SB1-P, respectively. The pH of extract from homogenized cells was 5.9 ± 0.2 (n = 4), characteristic of vacuolar pH (15).

Photosynthetic CO2 fixation at 9 μM CO2 was maximal from pH 6 to 7.5, with a sharp decrease under more alkaline conditions (data not shown). Gross photosynthesis by COT-PA cells at 10 mm total inorganic carbon saturated at about 300 μE/m²·s, with a maximum photosynthetic rate of 120 μmol CO2/mg Chl·h (data not shown). CO2 fixation in the dark was 8% of that in bright light. Based on these results all other experiments were carried out at pH 7.0 and 300 to 350 μE/m²·s unless otherwise specified.

CO2 Fixation

Photosynthetic CO2 fixation rates by healthy cotton cells (COT-PA and COT-P) were 100 to 130 μmol C/mg Chl·h at 10 mm inorganic carbon (pH 7), 30°C. Rates for SB-P soybean cells tended to be lower (60–90 μmol C/mg Chl·h), while rates for SB1-P soybean cells were higher (130–170 μmol C/mg Chl·h). Despite the variations in gross photosynthesis, O2 inhibition was not statistically different between the four cell lines. The amount of 14CO2 fixed at 300 μL/L CO2 in 50% compared to 2% O2 was 61% for COT-P cells, 14CO2 fixed at 300 μL/L CO2 in 50% compared to 2% O2 was 61% for COT-P cells, 62% for COT-PA cells, 71% for SB-P cells, and 69% for SB1-P cells. The CO2 response of photosynthesis by cotyledon COT-PA and soybean SB1-P cells at 21°C and 30°C showed apparent Michaelis-Menten kinetics, with KI/2(CO2) values of 37 ± 3 μM and 39 ± 3 μM, and Vmax values of 115 and 117 μmol C/mg Chl·h, respectively (Fig. 1).

CO2-Fixing Enzymes

RuBP carboxylase in cotton (COT-PA and COT-P) and the SB1-P soybean cell line was 93 to 94% activated in bright light (Table 1) with activation saturating at about 250 μE/m²·s (Fig. 2). However, in soybean line SB-P the enzyme was only 56% activated at 350 μE/m²·s. The initial activity of RuBP carboxylase in SB1-P cells increased by a factor of 2.5 as the light intensity increased from 10 to 200 μE/m²·s, approaching the total activity. Total activity was constant over the range of light intensities used. In contrast, the initial activity of RuBP carboxylase in the SB-P cells, while increasing with increasing light intensity, remained at only 40% of the total activity even at 1000 μE/m²·s. The total activity was about the same as in SB1-P cells and was also constant in the light. PEP carboxylase activity in the four cell lines ranged from 19 to 22 μmol C/mg Chl·h.

Total RuBP carboxylase activity in dark-adapted SB1-P soybean cells, measured after incubating the extract with CO2 and Mg2+, decreased 60% after 2 h in the dark (Table II). Initial activity decreased to 40% of the activity in the light.

**Figure 1.** CO2 response curve of soybean cells grown at 5% CO2 (SB1-P) and cotton cells grown at 660 μL/L CO2 (COT-PA). Cells were preilluminated for 10 min in flasks flushed with 21% O2, balance N2 in 30 mm Mops (pH 7.0), 30°C in the presence of carbonic anhydrase. Reactions were initiated with NaHCO3.
Table I. Ribulosebisphosphate and PEP Carboxylase Activities in Cell Suspension Cultures

<table>
<thead>
<tr>
<th>Cell Line</th>
<th>RubBP Carboxylase</th>
<th>PEP Carboxylase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial/Total</td>
<td>µmol CO₂/mg Chl·h</td>
</tr>
<tr>
<td>Cotton (COT-PA)</td>
<td>151 ± 3*</td>
<td>162 ± 2</td>
</tr>
<tr>
<td>Cotton (COT-P)</td>
<td>110 ± 4</td>
<td>114 ± 4</td>
</tr>
<tr>
<td>Soybean (SB-P)</td>
<td>86 ± 3</td>
<td>156 ± 1</td>
</tr>
<tr>
<td>Soybean (SB-1-P)</td>
<td>244 ± 3</td>
<td>262 ± 3</td>
</tr>
</tbody>
</table>

* Three samples from the same batch of cells were illuminated in each case.

Figure 2. Light activation of RubBP carboxylase in photoautotrophic soybean cells. Cells were illuminated for 5 min at 22 to 24°C in 30 mm Mops (pH 7.0) prior to homogenization in liquid N₂ in 1 mm PMSF, 1 µM leupeptin, and 1 µM antipain. For total activity the extract was incubated in 20 mm NaHCO₃, 20 mm MgCl₂, and 0.1 mm 6-P-glucuronate. (●) SB-P soybean cells, total activity; (▲), SB-1-P soybean cells, total activity; (◇), SB-1-P soybean cells, initial activity.

The cotton cell lines (COT-PA and COT-P) and soybean cell lines (SB-P and SB-1-P) showed a similar distribution of ¹⁴C label among the different fractions separated by ion exchange chromatography, with about one-third of the label in organic acids and sugars monophosphates, one-third in amino acids, and the remaining label nearly equally divided among the PGA, sugar bisphosphate, neutral, and insoluble fractions (Table III). In a separate experiment, the ¹⁴C-labeled insoluble material in COT-PA was shown to be at least 95% starch by digestion with α-amylase. In a ¹³CO₂ pulse-chase experiment with COT-PA cells at 335 µL/L CO₂, 21% O₂, balance N₂, about 35% of the ¹⁴C label was initially in the amino acid fraction. Labeled amino acids increased over 2 min of the chase and then remained relatively constant (Fig. 3). Thin-layer chromatographic analysis of this fraction from a separate experiment showed the initial distribution of ¹⁴C to be 69% Gly, 12% Ser, 11% Ala, 7% Asp, and 0.4% Glu. After a 15 min chase the ¹⁴C-label in Ser, Ala, and Glu had increased to 30, 20, and 7%, respectively, while Gly and Asp decreased to 38 and 6%, respectively. No other labeled amino acid was detectable in either case.

Prior to the chase, about 37% of the ¹⁴C was in the fraction containing organic acids and sugar phosphates, and the label in this fraction decreased continuously over the 15 min chase period (Fig. 3). Purification of this fraction by HPLC showed that the majority of label (85%) eluted in the void volume.
with 12% of the label coeluting with malate. After a 15 min chase, the percentage label in the void volume had decreased substantially, with the absolute amount in malate increasing slightly (from 6.6–7.9 nCi per 10 μg Chl). The labeled material in the void volume consisted of two compounds that eluted in the region of sugar monophosphates on a Whatman Partisil SAX HPLC column. The labeled compound coeluting with malate was verified as malate by decarboxylation with malic enzyme. Product analysis by HPLC showed no labeled malate remaining; however, 20% of the counts were retained in pyruvate. This indicates that the malate had been multiply labeled, or that some of the label at C₄ of malate had been scrambled with C₅ by fumarase.

**Inorganic Carbon**

Short-term photosynthesis of COT-PA cells in the presence and absence of external carbonic anhydrase exhibited a lag in the rate of ^14C-incorporation for the first 30 s after the addition of [^14C]NaHCO₃ (Fig. 4). Upon the addition of carbonic anhydrase the lag vanished, implying that CO₂, not HCO₃⁻, is the major form of inorganic carbon that penetrates these cells.

A time course of inorganic and organic carbon accumulation in the COT-PA cell line showed that the organic carbon (acid stable counts) increased linearly while the inorganic carbon (acid labile counts) was essentially constant from 15 to 105 s (Fig. 5). The intracellular concentration of CO₂ was calculated to be about 10 μM in the presence of 9 μM external CO₂, based on a cellular volume (³H₂O minus [¹⁴C]sorbitol space) of 3.37 mL/mg Chl.

**Carbonic Anhydrase**

Carbonic anhydrase levels in all four cell lines were examined both by immunoblotting and a standard biochemical
assay in veronal buffer. The antibody for the enzyme from spinach leaf was found to cross-react with proteins in leaf extracts from cotton and soybean. Both soybean cell lines tested positive for carbonic anhydrase, but no carbonic anhydrase was found in cotton cells grown at 5% CO₂ (COT-P) or at ambient CO₂ (COT-PA) (Table IV). We estimated that the cotton cells contained less than 1/200th of the carbonic anhydrase activity present in a spinach leaf. Activity in both spinach leaves and soybean SB1-P cells was in the soluble fraction, and was not associated with the membranes. Activity was completely inhibited by 10 μM ethozalomamide or by steaming the extract for 3 min.

**DISCUSSION**

This research was initiated in an attempt to determine any features distinguishing carbon metabolism in the photoautotrophic cells that grow at low CO₂ compared to cells that grow at only high CO₂. The results reported here show that photosynthetic carbon metabolism is identical insofar as it was investigated. Differences in any long-term effects, such as starch degradation or export of organic carbon, were not examined.

Many of the characteristics of the photoautotrophic cell suspension cultures examined here are very similar to those found in C₃ leaves and leaf cells. These cells are therefore potentially a useful model for photosynthesis in leaves. These results contrast with previous reports of carbon metabolism in a number of different photoautotrophic cell cultures, all of which contained much lower levels of RuBP carboxylase, a high fraction of ¹⁴C-labeled C₃ products, or both lower RuBP carboxylase activity and more C₄ acids (12, 13, 16, 18).

The CO₂ response of low CO₂-requiring cotton cells and high CO₂-requiring soybean cells demonstrated that most of the CO₂ was fixed by RuBP carboxylase (Fig. 1). The half-saturating level of CO₂, 40 μM at 21% O₂, was similar to that reported for soybean leaf cells (24). The presence of any inorganic carbon concentrating mechanism would have decreased this value, so it is unlikely that these cells concentrate CO₂ internally.

The ratio of RuBP carboxylase to PEP carboxylase (Table I) was similar to that in leaf tissue and much higher than ratios reported for other photoautotrophic cell cultures, where RuBP carboxylase was, at most, twofold higher than PEP carboxylase (8, 20). In the previous studies with photosynthetic cell cultures it was proposed that the low levels of RuBP carboxylase present were analogous to the low levels observed in developing leaves (13, 32). The reason for the difference in RuBP carboxylase levels in the different cell lines is unknown, but it is evident that all four of the cell suspensions studied in detail here are much more like cells in mature leaves.

RuBP carboxylase showed normal light activation at air levels of CO₂ in three of the four cell suspension cultures studied (Table I; Fig. 2). However, in the SB-P soybean cell line, RuBP carboxylase was only approximately half activated at high light. The enzyme itself appeared to be normal in that it could be fully activated with Mg²⁺ and CO₂, and the oxygen inhibition of CO₂ fixation was the same as in the other cell lines. In the dark, total RuBP carboxylase activity decreased in soybean cell line SB-P (Table II), suggesting that these cells synthesize the dark inhibitor, carboxyralbinol-1-phosphate, as found in soybean leaves (25).

The distribution of ¹⁴C-label also supports the major role of RuBP carboxylase in fixing carbon in these cell suspension cultures. As shown in Table III and Figure 3, the vast majority of the label is in products typical of C₃ photosynthesis. A relatively small amount of label was in malate, which turned over very slowly. This is in contrast to the results reported for several other photoautotrophic cell suspensions, all of which had a much higher proportion of the label in C₄ compounds (12, 13, 16, 18).

Photorespiration is a key indicator of C₃ photosynthesis and was found to occur in the suspension cell cultures. Significant levels of ¹⁴C-labeled glycine and serine were produced during photosynthetic CO₂ fixation. Also, the magnitude of O₂ inhibition of CO₂ fixation was similar to that seen in leaves of C₃ plants (5). This was not unexpected because the majority of the CO₂ is assimilated by RuBP carboxylase as shown by the distribution of ¹⁴C-label. Based on the kinetic properties of RuBP carboxylase in soybean leaf cells (24) it is likely that no photorespiration occurred under the elevated CO₂ conditions used for growth of the high CO₂-requiring cell suspension cultures.

Dark respiration in the cell suspensions was found to be variable. This may reflect, in part, the health of the cells or the stage of the growth cycle (22). Dark respiration rates were generally higher than the 6 to 7 μmol C/mg Chl·h reported for a soybean leaf (19). Similarly, a 7- to 10-fold higher rate of oxygen uptake has been reported for photoautotrophic cells compared to cells isolated from a tobacco leaf (29). This increase in respiration may reflect a more rapid cell division in the cell suspension, as is seen in developing leaves where the values decrease from about 100 μmol C/mg Chl·h to 11 μmol C/mg Chl·h in unfolding to mature leaves (6). However, the cell suspensions were grown under continuous light and the contribution of mitochondrial respiration in the light remains unclear.

Historically, high CO₂ has been necessary to support photoautotrophic cell growth. It was suggested (16) that any cell suspensions capable of growing at low CO₂ must have an inorganic carbon concentrating mechanism similar to that found in algae. As shown here, the cotton cell line which grew at 660 μL/L CO₂ did not possess a CO₂ concentrating mechanism. The lack of an inorganic carbon pump is shown most
pump directly into Chlamydomonas reinhardtii, which does have a CO₂ concentrating mechanism, and the absence of CO₂ accumulation in a Chlamydomonas mutant with a deficiency in the CO₂ concentration mechanism (28). Further, there is not a bicarbonate pump since the cells required CO₂, not HCO₃⁻, for photosynthesis (Fig. 4). Likewise, the magnitude of the oxygen inhibition of photosynthesis described above would not occur if the internal concentration of CO₂ were elevated.

One point that has been clarified by the results presented here concerns the possible role of carbonic anhydrase in aiding cells to grow at low CO₂. It was reported that photoautotrophic cells grown at 1% CO₂ had less than 10% of the carbonic anhydrase normally present in leaves (29), suggesting that the inability of the cells to grow at low CO₂ was due at least in part to the lack of the enzyme. However, the low CO₂ requiring cotton cells studied here contained no detectable carbonic anhydrase by either the standard biochemical assay or by an immunological probe of soluble cell extracts (Table IV).

The role of carbonic anhydrase in leaves has remained a source of speculation. The most commonly proposed roles are in regulating pH, in supplying CO₂ to RuBP carboxylase, or in aiding diffusion by increasing the CO₂ gradient (21). The enzyme is clearly not required for maximal photosynthesis in the photoautotrophic cotton cells that grow at low CO₂. More significantly, the CO₂ response of the cell lines that did and did not contain the enzyme are identical (Fig. 1). It is therefore unlikely that carbonic anhydrase plays a major part at the chloroplast level in either supplying CO₂ or removing H⁺. These results suggest that any requisite role is in dealing with transitory changes not found under the growth conditions of these cells, or that the enzyme is necessary only when the morphology of the leaf makes diffusion more problematic.

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LITERATURE CITED

PHOTOSYNTHETIC CARBON METABOLISM IN PHOTOAUTOTROPHIC CELLS


