Photorespiration and Internal CO₂ Accumulation in Chara corallina as Inferred from the Influence of DIC and O₂ on Photosynthesis

FRANÇOIS BRECHIGNAC and WILLIAM J. LUCAS
Botany Department, University of California, Davis, California 95616

ABSTRACT
An O₂ electrode system with a specially designed chamber for 'whorl' cell complexes of Chara corallina was used to study the combined effects of inorganic carbon and O₂ concentrations on photosynthetic O₂ evolution. At pH = 5.5 and 20% O₂, cells grown in HCO₃⁻ medium (low CO₂, pH ≥ 9.0) exhibited a higher affinity for external CO₂ (Kₐ(CO₂) = 40 ± 6 micromolar) than the cells grown for at least 24 hours in high-CO₂ medium (pH = 6.5), (Kₐ(CO₂) = 94 ± 16 micromolar). With O₂ ≤ 2% in contrast, both types of cells showed a high apparent affinity (Kₐ(CO₂) = 50 – 52 micromolar). A Warburg effect was detectable only in the low affinity cells previously cultivated in high-CO₂ medium (pH = 6.5). The high-pH, HCO₃⁻-grown cells, when exposed to low pH (5.5) conditions, exhibited a response indicating an ability to fix CO₂ which exceeded the CO₂ externally supplied, and the reverse situation has been observed in high-CO₂-grown cells. At pH 8.2, the apparent photosynthetic affinity for external HCO₃⁻ (Kₐ(HCO₃⁻)) was 0.6 ± 0.2 micromolar, at 20% O₂. But under low O₂ concentrations (<2%), surprisingly, an inhibition of net O₂ evolution was elicited, which was maximal at low CO₂ concentrations. These results indicate that: (a) photorespiration occurs in this alga and can be revealed by cultivation in high-CO₂ medium, (b) Chara cells are able to accumulate CO₂ internally by means of a process apparently independent of the plasmalemma HCO₃⁻ transport system, (c) molecular oxygen appears to be required for photosynthetic utilization of exogenous HCO₃⁻: pseudocyclic electron flow, sustained by O₂ photoreduction, may produce the additional ATP needed for the HCO₃⁻ transport.

The investigations dealing with the interactions between photorespiration and photosynthesis in aquatic photosynthetic organisms of the C₃ carboxylation type have focused mostly on microalgae. In contrast to high-CO₂-grown cells, microalgae cultured under low-CO₂ conditions appear to develop a CO₂-concentrating mechanism. This mechanism raises the apparent affinity of photosynthesis, for external DIC, to levels far exceeding the carboxylase affinity for CO₂ (1–4, 11, 20, 30). Consequently, the oxygenase activity of the Rubisco enzyme in low-CO₂-grown cells is inhibited, and they do not exhibit the general features associated with the occurrence of photorespiration, as deduced from the effects of O₂ concentration (8, 21, 28, 29, 31). In addition, since most of these cells appeared to be able to photosynthetically use exogenous HCO₃⁻, the CO₂ concentrating mechanism has been related to the operation of an active HCO₃⁻ pump located either in the plasmalemma (2, 11, 18) or in the inner chloroplast envelop (20). However, this HCO₃⁻ transport model cannot explain all the experimental data, and as a consequence, some authors have postulated the existence of a CO₂ transport system (1, 17).

The plasticity of the photosynthetic and photorespiratory patterns exhibited by aquatic macrophytes when grown in various culture conditions, has been recently stressed (5). Although there is a limited amount of information available on these aquatic macrophytes, it would appear that in some species, the state of reduced photorespiration may be attributed to the operation of a HCO₃⁻ accumulation system working in association with carbonic anhydrase activity (27), in a manner similar to that in some of the microalgae.

The little information is available concerning the macroalgae. Although Chara corallina has been extensively used to study the HCO₃⁻ transport phenomenon from a photosynthetic (12) as well as an ultrastructural viewpoint (24), controversy still exists as to the actual DIC species being transported across the plasmalemma. Furthermore, experiments relating to the presence or absence of a DIC concentrating mechanism have not yet been conducted.

In the present paper we report on studies in which we examined the combined influence of DIC and O₂ concentration on the photosynthetic rates exhibited by high- and low-CO₂-grown Chara cells. These results establish both the occurrence of photorespiration and a putative CO₂ concentrating mechanism in Chara corallina.

MATERIALS AND METHODS

Plant Material and Culture Conditions. Male cultures of Chara corallina Klein ex Wild., em. R. D. W. (= Chara australis R. Br.) were grown in 60 L containers in solutions of 'Chara pond water' (CPW: 1.0 mM NaCl, 0.2 mM KCl, 0.2 mM CaSO₄). Low-CO₂-grown cells were cultured in the presence of 1.0 mM NaHCO₃, and since stagnant conditions were employed, the bulk pH value ranged from 9 to 10.5, during photosynthetic activity. High-CO₂-grown cells were cultured in the above-mentioned conditions, except that a 4% CO₂ stream (balanced with air) of approximately 1 to 2 L h⁻¹ was bubbled through a sintered glass in a glass exchange column (height, 60 cm; diameter, 5 cm) placed vertically in the center of the culture tank. The exchange column prevented direct contact between the CO₂ and the Chara cells and also created a gentle convection stream within the culture medium, ensuring homogeneous repartition of the high CO₂ concentration. Under these high CO₂ conditions, the culture
medium was buffered with 3.0 mM Mes, and the pH value ranged from 5.5 to 6.5 during photosynthetic activity. In some experiments we also used cells obtained from short-term cultures. Here, plant material grown in control (HCO₃⁻, low CO₂) cultures was cut and transferred to 4-L beakers containing solution being bubbled with 4% CO₂. Provided a minimum culture period of 24 h was employed, these cells exhibited the same photosynthetic behavior as those grown for long-term periods in larger containers.

Artificial light from fluorescent lamps (cool-white, Lifeline; Sylvania, Seneca Falls, NY) provided a photon flux density at the solution surface of 115 μmol photon·m⁻²·s⁻¹ (measured using a Quantum Sensor, Licor, LI-190 SB). The photoperiod was 14 h-light and 10 h-dark.

Preparation of Chara `Whorl` Cells. The photosynthetic pattern of Chara cells was found to be very sensitive to perturbation caused by improper handling and physical treatment. Such a sensitivity has already been reported (15). These perturbations were reduced to a minimum by performing the experiments immediately after removing cells from the cultures. For this purpose, apical shoot axes were carefully removed from the culture tank; the selected mature branch cell complex (whorl) attached to the 2nd to the 5th node was carefully cut from the neighboring intermodal cells with sharp scissors. The peripheral (terminal) cells of each branch (5–7 branches per node) were cut in order to shape the branch complex with an approximate diameter of 5 cm, for proper loading into the measurement chamber. The excision and pruning of each whorl cell complex was carried out in freshly prepared CPW. Using this protocol, we found that freshly cut and prepared cells exhibited the fastest rates of photosynthetic O₂ evolution.

DIC Solutions. Solutions were prepared immediately before use. CPW free of CO₂ was first obtained by purging the CPW with CO₂-free air for 1 h. For experiments performed under 20% O₂, inorganic carbon was then added and the pH adjusted to 8.20 ± 0.15 for CO₂-containing solutions, and to 8.0 ± 0.15 for HCO₃⁻ containing solutions, with H₂SO₄ (0.1 and 0.01 N). The correct amounts of H₂SO₄ were previously determined to allow for rapid pH adjustment.

For experiments performed under low O₂ concentrations (≤2%), 100 ml of CO₂-free CPW was bubbled with pure N₂ for 15 min. Inorganic carbon was then added and the pH adjusted as previously described; during these manipulations, the neck of the flask was continuously flushed with a stream of pure N₂ to minimize atmospheric O₂ contamination.

We stress here that in the present study, buffers were not used because of their interaction with the uptake of inorganic carbon, particularly at high pH (14, 23).

Experimental Device. Because of the sensitivity of Chara cells to perturbation, we constructed a special O₂ electrode system having a Plexiglas chamber designed to hold whorl cell complexes with a minimum of disturbance to the cells (Fig. 1). The experimental chamber featured a recessed magnetic stirring bar (avoiding contact between the stir bar and the Chara cells), a small chamber volume (12 ml) for improved O₂ sensitivity, overall uniform illumination of the material, and two ports to permit solution changes. Experiments were performed under fast-stirring conditions, the rate being selected in the region where polarographic O₂ measurements became insensitive to the stirring speed. The O₂ partial pressure in the chamber was measured using a YSI 4004 Clark O₂ probe (YSI Co., Inc., Yellow Springs, OH). Saturating illumination for photosynthesis was found to occur at 100 μmol photon·m⁻²·s⁻¹; all experiments were then performed at 150 μmol photon·m⁻²·s⁻¹ which did not introduce problems due to photoinhibition. The light source was a Leitz Pradovit projector (model C1500, 150 W quartz-iodide lamp, E. Leitz, Raleigh, NJ), used with neutral density filters (Balzers, type no. 106613, PTR Optics Corp., Waltham, MA). Infrared irradiation was removed using a 10 cm-long water filter and all experiments were conducted in a thermostated room at 25 ± 2°C.

Experimental Procedures. Two Chara whorl cell complexes were carefully loaded into the chamber, which was then continuously illuminated. An investigation of the DIC concentration effect on photosynthesis always began by pretreating the cells in CO₂-free CPW and waiting for complete depletion of available CO₂, a condition assumed to be reached when the rate of O₂ evolution approached zero. The period necessary to establish this condition ranged from 3 to 30 min at 20% O₂, but was longer (up to 1 h) at low O₂ concentrations (O₂ contamination, which was leaking across the Teflon membrane from the electrolyte of the probe, was probably responsible). Thereafter, the DIC concentration was increased, with the chamber being rinsed several times between each measurement, and O₂ evolution was recorded over a 10 to 25 min period. Steady state rates of photosynthesis were reached in this range of time, with the O₂ concentration being maintained either at ±2%, or between 0 and 2%.

We also conducted CO₂ depletion experiments. Following a DIC concentration study (at 20% O₂), the experimental chamber was rinsed (four times) with CO₂-free CPW before introducing a solution having a low CO₂ concentration (20–60 μM, initial pH = 5.5). The depletion of CO₂ was recorded by the decrease of net O₂ evolution for periods of up to 60 min. Relatively small amounts of CO₂ were used to avoid a buildup of the O₂ concentration in the chamber and to reach a final pH value not exceeding 6.

RESULTS

Chara Photosynthetic Characteristics at pH = 5.5. Using our new experimental system, we found that low-CO₂-grown Chara cells (stagnant alkaline pH culture conditions, 1.0 mM HCO₃⁻) exhibited interesting photosynthetic characteristics. Figure 2 shows the influence, at pH = 5.5, of CO₂ concentration on the rate of net photosynthetic O₂ evolution. Net photosynthesis saturated at 300 μM CO₂, with maximal rates of approximately 200 pmol O₂·cm⁻²·s⁻¹ (about 88 μmol O₂·mg Chl⁻¹·h⁻¹). These cells exhibited a high apparent affinity for exogenous CO₂, as evidenced by the low CO₂ concentration required for half-maximal rate; the Km(CO₂) was approximately 40 μM. In contrast, high-CO₂-grown Chara cells (Fig. 3) required between 500 and 1000 μM CO₂ to attain saturation, with the maximal photosynthetic rate in these cells being approximately 160 pmol O₂·cm⁻²·s⁻¹. We attribute the difference between the maximal rates in low- and high-CO₂-grown Chara cells to a slightly lower Chl concentration (on a surface basis) in the high-CO₂-grown cells (data not shown). However, high-CO₂-grown cells had a lower apparent affinity for exogenous CO₂; the Km(CO₂) was approximately 90 μM.

Influence of O₂ Concentration on Photosynthesis at pH = 5.5. The influence of low (≤2%) and atmospheric (20 ± 2%) concentrations of O₂ on the photosynthetic response curves for exogenous CO₂ (pH = 5.5) are shown for low-CO₂-grown (Fig. 4) and high-CO₂-grown (Fig. 5) Chara cells. Clearly, low-CO₂-grown cells were nearly insensitive to a reduction in the level of O₂. In contrast, photosynthesis in high-CO₂-grown cells was clearly stimulated when the O₂ concentration was lowered.

Figure 6 presents a summary of the stimulation of photosynthesis at low O₂, compared to atmospheric O₂ levels (Warburg effect), that we obtained when we investigated the effect of CO₂ concentration on low- and high-CO₂-grown cells. In high-CO₂-grown cells, the Warburg effect increased as the CO₂ concentration was reduced, except for very low CO₂ concentrations. The
average apparent affinities for exogenous CO₂, obtained under low and atmospheric O₂ levels, for a number of different samples of low- and high-CO₂-grown Chara cells, are presented in Table I. The difference observed in the Kₐ(CO₂) between the low and 20% O₂ level experiments for low-CO₂-grown cells is not statistically significant.

Photosynthetic CO₂ Depletion Experiments Conducted at pH = 5.5. Photosynthetic CO₂ depletion of the medium (initial pH = 5.5) surrounding the cells was followed using both low- and high-CO₂-grown Chara cells. These experiments were conducted at the completion of the CO₂ concentration studies, and were performed in order to compare the amount of CO₂ externally supplied to the amount of CO₂ fixed photosynthetically. The CO₂ fixed was deduced from the integration of the rate of O₂ evolution as a function of time, assuming a photosynthetic ratio of 1. Figure 7 (inset) illustrates a typical exponential decrease in the rate of O₂ evolution versus time. This exponential decrease was confirmed by plotting the logarithm of the rate of O₂ evolution-against time (Fig. 7). From this form of analysis, we obtained the appropriate exponential functions. In this way we could calculate, on the same mathematical basis, all the integrals of each different function. Table II gives the results of these integrations, in which we estimated the amount of carbon fixed, in comparison to the amount of carbon externally supplied, along with the Kₐ(CO₂) values which were determined in the respective preceding experiments.

We found that in low-CO₂-grown Chara cells, with high apparent affinities for exogenous CO₂ (Kₐ(CO₂) = 34–40 μM), the carbon fixed always exceeded the externally supplied DIC. Consequently, some internal CO₂ must have been present in these cells at the beginning of the CO₂ depletion experiment. Alternatively, the internal CO₂ might have been raised by simple diffusion during the preceding investigation in which high CO₂ concentrations were employed. This maximal putative amount of internal DIC, which would have resulted from diffusion, can be

**FIG. 1.** Plexiglas chamber for measuring O₂ evolution in whorl cell complexes of C. corallina. This system featured a recessed magnetic stir bar (A), an O₂ electrode (B), an inlet (C), and outlet (D) port with a Teflon valve (E), a magnetic stirrer (F), and a removable lid (G) hermetically closed the chamber by means of an O-ring (H).

**FIG. 2.** Influence of CO₂ concentration on the rate of O₂ evolution in low-CO₂-grown cells of C. corallina. Each symbol represents a different experiment, conducted at pH 5.5 and over a range of CO₂ concentrations, on two whorl cell complexes which were grown in HCO₃⁻ medium (low CO₂, pH > 9).

**FIG. 3.** Influence of CO₂ concentration on the rate of O₂ evolution in high-CO₂-grown cells of C. corallina. Details as in Figure 2.
estimated by assuming a total inefficiency of the four rinses (with CO₂-free CPW) in removing some internal CO₂ along with the external CO₂. However, even after deduction of the corresponding values, the internal CO₂ present remained significant (Table II, "internal carbon fixed," values in parentheses).

In contrast, photosynthesis by the high-CO₂-grown Chara cells (low apparent affinity for CO₂: K₁/₂(CO₂) = 80–98 μM) stopped before removal of all the external CO₂, as shown by the "unfixed external CO₂" values (Table II).

**Influence of O₂ and DIC Concentration on Photosynthesis at pH = 8.2.** Typical examples of the influence of DIC concentrations on photosynthesis at low (<2%) and atmospheric (20%) O₂ levels are presented in Figure 8. At pH = 8.2, HCO₃⁻ is the major DIC species present in solution. Thus, the observed photosynthetic response to changes in DIC concentration (pH = 8.2, 20% O₂) can be described as follows: Vₘₐₓ(HCO₃⁻) = 107 ± 11 pmol O₂·cm⁻²·s⁻¹ and K₁/₂(HCO₃⁻) = 0.6 ± 0.2 mm. Surprisingly, compared to 20% O₂, low O₂ tensions caused an inhibition of photosynthesis. This inhibition was quite reproducible (Fig. 9),

**DISCUSSION**

**Kinetic Properties of Chara Photosynthesis with Exogenous DIC.** Our maximum rates of photosynthesis, obtained with Chara branch cells exposed to 20% O₂ and CO₂ as the main form of DIC (pH 5.5), were very close to those reported in a similar study by Price et al. (24). With our tissue, the apparent affinity for CO₂ (K₁/₂(CO₂) = 40 μM) was higher than the value reported by these workers (76 μM). Nevertheless, Price et al. (24) recognized that a higher affinity could probably have been obtained by using a more rapid stirring rate, which would have reduced the extent of the unstirred layer between the bulk medium and the cell surface. However, they reported no difference between the cells grown in alkaline (pH = 9.3) and those grown in slightly acidic (pH = 6.8) conditions, a feature in apparent contradiction with the clearly distinct affinity for CO₂ found in the present study (Table I). The probable explanation is that the Chara cells used by Price et al. (24) were all grown under low CO₂ conditions, corresponding to an atmosphere-equilibrated CO₂ concentration, at most, whereas our acid cultures were CO₂ enriched.

The photosynthetic characteristics of alkaline-grown cells ob-

**Fig. 4.** Influence of O₂ concentration on the photosynthetic response to CO₂ in low-CO₂-grown cells of C. corallina. The rates of O₂ evolution were measured at pH = 5.5 under 20% O₂ (●) and ≤2% O₂ (O).

**Fig. 5.** Influence of O₂ concentration on the photosynthetic response to CO₂ in high-CO₂-grown cells of C. corallina. The rates of O₂ evolution were measured at pH = 5.5 under 20% O₂ (●) and ≤2% O₂ (O).

**Fig. 6.** Effect of low O₂ concentration on photosynthesis in high- and low-CO₂-grown cells of C. corallina at various concentrations. The stimulation at low O₂ (≤2% O₂) is expressed as a percentage of the rate measured under 20% O₂. (Values represent Mean ± SD n ≥ 3).

Table I. Apparent Photosynthetic Affinity for Exogenous CO₂ in High-CO₂- and Low-CO₂-grown Chara Cells at Atmospheric (20%) and Low (<2%) O₂ Concentrations

<table>
<thead>
<tr>
<th>O₂ Concentration</th>
<th>K₁/₂(CO₂) (μM ± SD)</th>
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<tbody>
<tr>
<td>≤2% O₂</td>
<td>52 ± 9 (4)*</td>
</tr>
<tr>
<td>20% O₂</td>
<td>94 ± 16 (9)</td>
</tr>
</tbody>
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*Mean ± SD; values in parenthesis indicate number of experiments performed.
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Table II. Comparison between the Amounts of Inorganic Carbon Supplied and Photosynthetically Fixed in Low- and High-CO2-grown Chara Cells

<table>
<thead>
<tr>
<th></th>
<th>High-CO2-Grown Cells</th>
<th>Low-CO2-Grown Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>$K_{1/2}$ (CO2 $\mu$M)</td>
<td>80  98  98</td>
<td>40  34  34</td>
</tr>
<tr>
<td>Carbon fixed (nmol CO2)</td>
<td>221  305  359  413  377  614  544</td>
<td></td>
</tr>
<tr>
<td>Carbon supplied (nmol CO2)</td>
<td>720  480  480  360  240  480  480</td>
<td></td>
</tr>
<tr>
<td>Unfixed external carbon (nmol CO2)</td>
<td>499  175  121</td>
<td></td>
</tr>
<tr>
<td>Internal carbon (nmol CO2)</td>
<td>53  137  134  64 (42) (89) (89) (19)</td>
<td></td>
</tr>
</tbody>
</table>

Fig. 7. Kinetics of O2 evolution during photosynthetic CO2 depletion studies on low- and high-CO2-grown cells of C. corallina. The decrease in net O2 evolution as a function of time (see inset) has been represented in a semilogarithmic plot for four experiments on low-CO2-grown cells (open symbols, solid lines) and three experiments on high-CO2-grown (closed symbols, broken lines). The high regression coefficients ($r^2 > 0.97$) indicate that the decrease in O2 evolution followed an exponential function.

Fig. 8. Influence of O2 concentration on photosynthesis in HCO3$^-$-grown (low CO2) C. corallina. O2 evolution was measured at pH = 8.2 in 20% (●) and ±2% O2 (○).

Fig. 9. Inhibition by hypoxia of HCO3$^-$-supported photosynthesis in HCO3$^-$-grown (low CO2) C. corallina. The inhibition at low O2 (±2%) is expressed as a percentage of the rate measured under 20% O2 (at the same DIC concentration).

The clear Warburg effect observed in high-CO2-grown cells of...
C. corallina (Fig. 5) is consistent with the operation of photorespiration in this species. In accordance with the model of competitive inhibition at the Rubisco level (9): (a) the stimulation of photosynthesis by low O2 tensions was CO2 sensitive, being maximal at low CO2 and disappearing at saturating CO2 concentrations (Fig. 6); (b) the apparent affinity of photosynthesis for CO2 was O2-sensitive in high-CO2-grown cells (Table I). Such a behavior has often been reported in high-CO2-grown microalgae (2, 21, 29), or in microalgal mutants (30), both of which have a reduced ability to concentrate CO2. The reduced stimulation of photosynthesis observed at 10 μM CO2 may be due to the inactivation brought about by low CO2 of either the catalytic site of Rubisco or the PSI1 complex.

Evidence for an Internal CO2 Concentrating Mechanism. Unlike high-CO2-grown cells, photosynthesis in low-CO2 grown (HCO3- medium, pH > 9) Chara cells was O2-insensitive when the experiments were performed under acidic conditions (pH = 5.5). The Warburg effect was absent (Figs. 4 and 6), and the apparent affinity of photosynthesis for CO2 was high and almost not affected by O2 (Km(CO2) = 40–50 μM, Table I). In microalgae, the kinetic characteristics of the Rubisco have been shown to be similar in high- and low-CO2-grown cells (4, 32). Hence, the reduced O2-sensitivity of photosynthesis has been explained on the basis of an internal CO2 concentrating mechanism, occurring in low-CO2 grown cells, which suppresses the potential oxygenase activity of the Rubisco (2, 3, 8, 10, 11). Thus, the absence of detectable photorespiration (as deduced from O2-insensitive photosynthesis) in low-CO2-grown Chara cells appears consistent with the hypothesis that these cells can also raise their internal CO2 above the external CO2 concentration.

Compared with the apparent affinity of photosynthesis for CO2, the lower affinity exhibited, in vitro, by the Rubisco extracted from some microalgae is another argument in favor of a CO2 concentrating mechanism (11, 32). Yeoh et al. (34) reported a value of 42 ± 4 μM for the Km(CO2) of Rubisco extracted from a Chara species, a value which was determined in the absence of O2. Hence, in the absence of a CO2 concentrating mechanism, intact Chara cells placed in 20% O2 should exhibit a Km(CO2) higher than 42 μM. Furthermore, the resistances limiting CO2 diffusion between the external medium and the site of CO2 fixation will also contribute to reducing the apparent photosynthetic affinity for CO2. However, our Km(CO2) for low-CO2-grown Chara cells remained low (40 ± 6 μM, Table I), even in 20% O2, a result which supports our hypothesis that a CO2 concentrating mechanism is involved in the photosynthesis of low-CO2 grown Chara cells to internally accumulate CO2 as also supported by our CO2-depletion experiments conducted in the presence of 20% O2 (Fig. 7). The carbon fixed exceeded the carbon externally supplied, a behavior which was not observed in high-CO2-grown cells (Table II). Such an excess in carbon fixed still remained even when we allowed for the maximal potential error introduced by the experimental protocol (see "Materials and Methods"). Since the investigation of the CO2 effect on photosynthesis (which was performed before a CO2 depletion study) began only after the cells had removed all the contaminating CO2, externally as well as internally (i.e. when O2 evolution completely stopped), these cells must have been able to accumulate some CO2 from the previously used acidic (pH = 5.5) bathing medium. This ability to accumulate CO2 appears to be independent of the presence of HCO3- in the external solution.

Many reports dealing with microalgae have emphasized that the internal CO2 accumulation was caused by the operation of an active HCO3- pump located in the plasmalemma (11, 18). In Chara cells, the presence of a plasmalemma-mediated HCO3- acquisition mechanism, driven by an ATP-dependent H+ extrusion, is well established (for a review, see Lucas [13]). The results presented here (Fig. 7; Table II) suggest that the CO2 accumulation mechanism in Chara, when activated, does not depend exclusively on this process.

The inhibition only occurred under limiting HCO3- concentrations (Fig. 9). This indicates an O2 involvement with one of the steps between the uptake of exogenous HCO3- and the final CO2 fixation step. Such an effect has already been reported in Anacystis nidulans, a HCO3-·user, where the rate of 14C-fixation was enhanced at 21% O2 compared with anoxic conditions (19, 21). In the work of Miyachi and Okabe (19), the stimulation only occurred under limiting HCO3- concentrations and under high light intensities, conditions similar to those used in the present study. It should be noted that in our experiments, inhibition of photorespiration by low O2 would raise the rate of CO2 fixation within the chloroplasts. However, this photosynthetic enhancement is masked (or prevented) by an inhibition, via the influence of the putative O2-sensitive step.

The ability is interesting to note that in low-CO2-grown Chara cells we observed an O2 stimulation only under exogenous HCO3- assimilating conditions. In addition, an electrophysiological interaction between O2 and the HCO3- transport system in Chara has been recently demonstrated (16). Thus, it appears that the HCO3- uptake process, per se, is facilitated by O2. This requirement may be an evolutionary adaption to the ecological habitat occupied by Chara, namely stagnant water, where HCO3- assimilation at alkaline pH values is often associated with super saturating dissolved O2. O2 may be involved in the biochemical process supplying energy to the transport system. It is likely that HCO3- acquisition in Chara is driven by ATP, whatever the mechanism (13; see also later section). A Mehler-type O2 photoreduction driving pseudocyclic photophosphorylation has been postulated to supply the HCO3- transport systems of Chondrus crispus (6, 7) and Scenedesmus (25) with ATP, and such a process may also be involved in Chara. This mechanism is at variance with the model proposed by Ogawa et al. (22), in which it was suggested that O2 interaction with ferredoxin (Fd) inhibits the DIC accumulation mechanism functioning in Anacystis, a process which they suggested is driven by cyclic photophosphorylation.

However, at present it is not clear why O2 photoreduction should have a higher efficiency (enhanced DIC accumulation of photosynthesis) when photosynthesis is HCO3- ·limited. Possibly, cyclic photophosphorylation might be occurring in the presence of saturating HCO3-, when NADP+ does not limit its reduction to NADPH at the reduced side of Fd. In this situation, O2 is not involved and no influence on the photosynthetic rate is expected. When the level of exogenous HCO3- limits photosynthesis, NADP+ regeneration becomes limiting and would result in competition with O2 for electrons at the Fd level. This may allow O2 photoreduction to drive pseudocyclic photophosphorylation, in aerobic conditions, and thereby sustain a higher rate of HCO3- uptake and fixation compared with hypoxic conditions.

The mechanism of HCO3- acquisition. It has been proposed that exogenous HCO3- enters the cell at alkaline pH by diffusion of CO2, rather than HCO3- transport (see Lucas [13] for a review of the relevant literature). In the extracellular CO2 production hypothesis, it was suggested that a plasmalemma-bound H+ translocating ATPase created a high CO2 partial pressure in the unstirred layer next to the wall of these giant cells (33). However, Lucas et al. (14) showed that fast-flowing solutions, which would remove the CO2 generated in the unstirred layer, caused an increase, rather than the predicted decrease, in photosynthesis. Our present results (Fig. 8) are also inconsistent with the extra-
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cellular CO₂ production hypothesis, in that our measured rates of O₂ evolution (thin boundary layer due to high stirring rate) were not lower than the ¹⁴CO₂ fixation rates obtained in stagnant solutions (12).

In a modified version of the above-mentioned hypothesis, Price et al. (24) and Price and Badger (23) suggested that the extracellular CO₂ partial pressure was created in the periplasmic space of the charasomes, a region which would be relatively insensitive to the rate of solution flow. This hypothesis does not appear to be supported by the data presented in Figure 8. In the presence of HCO₃⁻, photosynthesis appears to saturate at about 3 mm DIC, with the maximum rate being 90 pmol cm⁻² s⁻¹. From the CO₂-fixation data in Figure 2, we can estimate that this Vₘ₉ would require an exogenous CO₂ concentration in the periplasmic space of, at most, 35 μM. Clearly, this value is still below the apparent K₉ (CO₂). Unless the energy demands become too great, it seems unlikely that the periplasmic/charasome CO₂-production model can explain why increasing DIC does not raise the external CO₂ concentration (and then photosynthesis) to values higher than 35 μM.

Finally, if CO₂ were being supplied to the chloroplasts through an extracellular CO₂ production mechanism, investigation of the DIC influence on photosynthesis should have exhibited a stimulation of photosynthesis, by low O₂ since we have established that photorespiration occurs in Chlamydomonas reinhardtii. However, as illustrated in Figures 8 and 9, we observed an inhibition of photosynthesis under low O₂ conditions.

Future experiments will utilize MS to investigate both the mechanism of O₂ involvement in DIC utilization and the extent to which the putative CO₂ concentrating mechanism raises the internal DIC level in Chara.  

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LITERATURE CITED