Photosynthesis and inorganic carbon utilization in *Pleurochrysis* sp. (Haptophyta), a coccolithophorid alga

Alvaro A. Israel*, Elma L. González**

Department of Biology, University of California Los Angeles, Los Angeles, California 90095-1606, USA

ABSTRACT: We studied and compared properties of inorganic carbon fixation for 2 coccolithophorid strains that differ in their capacity to calcify; namely high calcifying (HC) *Pleurochrysis* sp. CCMP 299 and low calcifying (LC) *Pleurochrysis* elongata CCAP 961/3. *Pleurochrysis* species are unicellular algae that, in nature and in culture, produce intracellular CaCO₃ encrusted structures (coccoliths). *Pleurochrysis* and other calcifying algae, are potential players in atmospheric CO₂ cycling and the maintenance of global carbon balances. In *Pleurochrysis* sp. and *P. elongata* (hereafter *Pleurochrysis*), photosynthesis was affected by increasing O₂ (from 1 to 21% in air), with 18% inhibition for LC cells and 9% for HC cells. The inhibition could be reversed by (1) decreasing the ambient O₂, (2) reducing the ambient pH (which rose in the medium, particularly for LC cells) and (3) by increasing the ambient inorganic carbon concentration. Carbonic anhydrase activity was detected in *Pleurochrysis*; HC cells having approximately 4 times more activity than LC cells. Inhibition of carbonic anhydrase by 0.25 mM acetazolamide (a non-membrane-permeating inhibitor of the enzyme) averaged 30% in HC cells and only 10% in LC cells. Calcium uptake measured for HC cells was 2.5 to 3.0 times higher in the light and 4 times higher in the dark than calcium uptake measured for LC cells. Rates of photosynthetic O₂ evolution were significantly higher for both strains at acidic pH (e.g. 5.0, containing about 90% CO₂) than at seawater pH (e.g. 8.0, containing about 1% CO₂), while at a basic pH (e.g. 9.0, virtually no CO₂ and about 50% HCO₃⁻) rates were still substantial for HC cells but extremely low for LC cells. These data indicate that HC cells in their natural environment are primarily HCO₃⁻ users. By comparing seawater CO₂ concentrations (i.e. 15 μM) with calculated K<sub>m</sub>(CO₂), the CO₂ concentration required for a half-maximal rate of photosynthetic O₂ evolution, for *Pleurochrysis* (51 and 37 μM for HC and LC cells, respectively) it follows that CO₂ must be concentrated intracellularly for effective photosynthesis in both strains. Thus, an adequate CO₂ supply depends on HCO₃⁻ utilization and concomitant calcification, particularly in HC cells.

KEY WORDS: Calcium uptake · Carbon utilization · Coccolithophorid · Photosynthesis · Rubisco

INTRODUCTION

Among the unicellular marine algae, coccolithophorids (Haptophyceae) are a widespread group of phytoplankters with distinct scales or plates of CaCO₃ (coccoliths) that, either partially or entirely, cover the cell in a loosely organized layer. Coccolithophorid algae remove large quantities of atmospheric CO₂ through photosynthesis and calcification and are, therefore, an important component of the global carbon cycle (Raven 1991, McConnaughey 1994), accounting for a substantial part of the ocean floor limestone sediments.

Inorganic carbon (C<sub>i</sub>) dissolved in seawater is mostly composed of high levels of HCO₃⁻ (ca 2100 μM) and low concentrations of CO₂ (ca 15 μM). Thus, not surprisingly, most of the microalgae (Dixon et al. 1987, Munoz & Merrett 1989) as well as the macroalgae (Beer 1994) are able to utilize HCO₃⁻ as the exogenous C<sub>i</sub> source for photosynthesis. Some microalgae can translocate HCO₃⁻ across the plasmalemma for intra-
cellular conversion to CO₂ (Dixon et al. 1987), while others convert HCO₃⁻ to CO₂ extracellularly with CO₂ readily diffusing into the cell (Badger et al. 1980). A few algae rely on diffuse entry or active uptake of CO₂ (Raven 1991, Badger 1985). The CO₂ is thus concentrated intracellularly, enhancing the levels of CO₂ at the site of fixation via ribulose 1,5-bisphosphate carboxylase/oxygenase (Rubisco). Carbonic anhydrase (CA) is associated with one of these 'biophysical C₄ concentrating mechanisms' since it catalyzes the reversible hydration of CO₂ (H⁺ + HCO₃⁻ ⇌ H₂CO₃ ⇌ H₂O + CO₂). This enzyme is localized in the chloroplast stroma and/or bound to the plasmalemma (Moroney et al. 1985).

Studies on transport and fixation of C₄ have largely focused on green microalgae and cyanobacteria. Among the coccolithophorid microalgae, *Emiliania huxleyi* has received the most attention because of its ecological importance in marine environments. Despite its putative role in the global carbon cycle, *E. huxleyi* is apparently limited by the CO₂ concentrations of seawater (Sikes et al. 1980, Nimer et al. 1992). Furthermore, it is known that *E. huxleyi* utilizes HCO₃⁻ for calcification and photosynthesis, although a low calcifying strain has shown poor affinity for HCO₃⁻ and preferentially uses CO₂ (Nimer & Merrett 1992, Sekino & Shiraia 1994).

If bicarbonate is a source of CO₂ for carbon fixation, it is also, simultaneously, a source of carbonate for mineralization (calcification) (see McConnaughey 1994). Apparently, the calcification reactions are important for sustaining high rates of photosynthesis in calcifying cells (Nimer & Merrett 1992, 1993). Conversely, if CO₂ is taken in across the plasmalemma, the acidotic calcification reactions may not be necessary for sustaining photosynthesis. Current models for C₄ acquisition in the coccolithophorid algae do not take into consideration a potential extracellular location of CA. Instead, they primarily focus on an internally localized (i.e. chloroplast) enzyme to boost HCO₃⁻ → CO₂ rates (Sikes & Wheeler 1982, Quiroga & González 1993).

The present investigation compares 2 strains of *Pleurochrysis* differing in their calcification capacity. The relationships among C₄ utilization, carbonic anhydrase, calcification and photosynthesis are explored. Ultrastructural (Brown & Romanovicz 1976) and cellular studies of calcification (Kwon & González 1994) have been conducted on *Pleurochrysis*, but no previous physiological work has been done to elucidate its mechanism of C₄ utilization.

**MATERIALS AND METHODS**

**Algae and growth conditions.** A high calcifying (HC) strain of *Pleurochrysis* sp. (CCMP299, Center for Culture of Marine Phytoplankton, Bigelow Laboratory, Maine) and a low calcifying (LC) *Pleurochrysis elongata* (CCAP961/3, Culture Collection of Algae and Protozoa, Scotland) were grown axenically at 15°C and pH 7.5 as described previously (Wainwright et al. 1992). Cultures used in subsequent experiments were harvested at 5 wk (Quiroga & González 1993). The 2 strains used in this study are hereafter called *Pleurochrysis*.

**Measurements of net photosynthesis.** Photosynthetic O₂ evolution was measured from 4.0 ml cell suspensions (averaging 4.7 ± 0.8 × 10⁵ cell ml⁻¹, n = 5 ± SD) with an O₂ electrode, liquid phase system (Hansatek Ltd, Kings Lynn, Norfolk, UK), set at 15°C and attached to a chart recorder. Usually, just before measurements were started, O₂ in the cell suspension was reduced to desired levels by gentle bubbling with N₂ for up to 30 s. Cells were allowed to photosynthesize under saturating photosynthetic photon flux (PPF) of 500 µmol m⁻² s⁻¹, PAR was provided by halogen lamps until rates of O₂ evolution were steady. Saturating PPF was established from measurements of net photosynthesis at various PPF levels created with neutral density glass filters.

**pH effects on photosynthesis.** The response of photosynthetic O₂ evolution to changes in the ambient (extracellular) pH was determined in 10 mM biological buffer (Sigma). A stock solution of MES (pH 5.0 and 6.0), Bis-TRIS (pH 6.5), HEPES (pH 7.0 and 7.5), TRIS-HCl (pH 8.0) or CAPS (pH 9.0) was injected into a cell suspension in 2 mM inorganic carbon (C₄) in the O₂ electrode chamber. Measurements were continued until stable rates of photosynthesis were recorded.

**Photosynthesis under carbon limitation.** CO₂-depleted cell suspensions were prepared as follows: a 30 ml culture sample was allowed to stand for 45 min at saturating PPF until the cells settled to the bottom of sterile plastic tubes. Cells were then retrieved with a pipette and resuspended in a CO₂-free artificial seawater medium (450 mM NaCl, 10 mM CaCl₂, 10 mM KCl and 30 mM MgSO₄) for an additional 30 min under illumination. This technique for cell collection was preferred because centrifugation apparently reduced the photosynthetic capacity of *Pleurochrysis*. C₄-dependent O₂ evolution was measured after NaHCO₃ (from freshly prepared stock solutions) was added to cells suspended in CO₂-free artificial seawater medium. The bicarbonate-free O₂ electrode system was closed, and the pH was set at 5.5 with MES, 7.5 with HEPES, or 8.5 with TRIS-HCl. After a stable baseline was attained, aliquots of the NaHCO₃ stock solution were injected into the electrode chamber to generate a range of C₄ concentrations. To prepare cells for measurements at low pH, the samples from both strains were treated with 1 N HCl and stirred for 3 to 4 min.
Chlorophyll a was extracted and measured according to Moran (1982); cells were collected by centrifugation from 1.0 ml cell suspensions and immersed in 1.0 ml N,N-dimethylformamide at 4°C in the dark for 24 h. The extracts were read at 663 and 647 nm with the spectrophotometer. Rates of non-enzymatic dehydration of HCO$_3^-$ to form CO$_2$ ($d$C$_{02}$/dt) were calculated according to Johnson (1982). PPF was measured with a LI-193S quantum sensor (LI-COR, Lincoln, NE). Statistical significance of results was determined by Student's t-test.

**RESULTS**

Low calcifying cells and high calcifying cells required similar PPF to achieve maximal rates of photosynthetic O$_2$ evolution, both about 200 pmol m$^{-2}$ s$^{-1}$ (Fig. 1). At 500 pmol m$^{-2}$ s$^{-1}$ (i.e. saturating PPF) net photosynthesis in LC cells averaged 13% higher (p < 0.05) than net photosynthesis in HC cells. At the ambient PPF for culture growth in the laboratory (75 pmol m$^{-2}$ s$^{-1}$) the content of chlorophyll a was 20% higher in HC cells than for LC cells (p < 0.01; Fig. 1). At 227 pmol O$_2$ at 15°C, the rates of net photosynthesis at pH 7.5 were 82% (p < 0.05) for LC cells and 91% (p < 0.05) for HC cells when compared to the rates of net photosynthesis attained at 2% O$_2$ (21.3 μM O$_2$; Table 1).

Fig. 1 P. elongata and P. elongata. Rates of net photosynthesis (%PS) as a function of photosynthetic photon flux (PPF) for low calcifying (○) cells and high-calcifying (□) cells. Measurements were conducted at 15°C, pH 7.5 and 2.0 mM C$_3$ (inorganic carbon). (n = 5 ± SD)
Table 1. Pleurochrysis sp. and P. elongata. Rates of net photosynthesis (NPS) in response to extracellular O2 concentration for low (LC) and high calcifying (HC) cells. Measurements were conducted at 15°C, 500 μmol m−2 s−1, pH 7.5 and 2.0 mM C, (inorganic carbon). (n = 8 ± SD)

<table>
<thead>
<tr>
<th>O2 (μM)</th>
<th>O2 in Air (%)</th>
<th>NPS (mmol O2 10^6 cell^1 h^1)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>LC</td>
</tr>
<tr>
<td>14.2−28.4</td>
<td>1.3−2.6</td>
<td>73.6 ± 6.7</td>
</tr>
<tr>
<td>198.6−255.4</td>
<td>18.4−23.6</td>
<td>60.1 ± 5.8</td>
</tr>
<tr>
<td>340.5−368.9</td>
<td>31.5−34.1</td>
<td>56.9 ± 4.7</td>
</tr>
</tbody>
</table>

Fig. 2. Pleurochrysis sp. and P. elongata. Example of an O2 evolution trace (redrawn) for low calcifying (LC, solid line) and high calcifying (HC, dashed line) cells which were initially bubbled with N2 + CO2 to reduce existing O2 levels to approximately 20 μM, and then illuminated (on) at pH 7.5 and 2.0 mM C, (inorganic carbon). After O2 inhibition was apparent, the CO2:O2 ratio was increased by adjusting the pH to 6.0 (see Fig. 3), or by increasing the C1 concentration to 2 mM C, (pH/C1).

increased to about 350 μM, rates of photosynthetic O2 evolution decreased, on average, by 23% (p < 0.01) for LC cells and by 13% (p > 0.05) for HC cells (Table 1).

Photosynthetic O2 evolution became inhibited as O2 levels increased within the electrode chamber (Fig. 2, Table 1). For both LC cells and HC cells, rates of photosynthesis at ≥250 μM O2 could be enhanced to the rates more typically observed at 5% O2 if the ambient pH was reduced to 6.0, or if C1 was added directly to the sample of photosynthesizing cells (Fig. 2).

Photosynthesis increased the ambient pH. The pH of cell suspensions that had been illuminated for 20 min was increased by 1.0 unit for LC cells and by 0.5 units for HC cells, and then stabilized (Fig. 3). Upward drifts of extracellular pH were correlated with illumination since light withdrawal led to a rapid downward shift of pH, and vice-versa. Moreover, the extracellular pH of 5 wk old cultures in 2 l flasks (after 6 h of illumination) averaged 8.9 ± 0.2 (n = 4 ± SD) and 8.2 ± 0.3 (n = 5 ± SD) for LC cells and HC cells, respectively. The photosynthetic O2 evolution of LC cells was stable from pH 5.0 through pH 7.0, decreased at pH 8.0, on average, by 55% (p < 0.01), and at pH 9.0 averaged 7% (p < 0.01) of rates at pH 5.0 (Fig. 4). The photosynthetic O2 evolution of HC cells was similar from pH 5.0 through pH 8.0 and at pH 9.0 averaged 53% (p < 0.01) of rates at pH 5.0 (Fig. 4).

Acetazolamide (2 mM) inhibited photosynthesis, on average, by 70% (p < 0.01) for both LC cells and HC cells (Fig. 5); however, the magnitude of the inhibition differed at lower AZ concentrations. For example, inhibition by 0.25 mM AZ averaged 10% (p < 0.05) in LC cells and 30% (p < 0.01) in HC cells (Fig. 5). Carbonic anhydrase activity of intact cells was 4 times greater for HC cells than for LC cells (p < 0.01; Fig. 6). Inhibi-
Acetazolamide (mM)

Fig. 5. *Pleurochrysis* sp. and *P. elongata*. Effect of acetazolamide on net photosynthesis (NPS) for low calcifying (○) cells and high calcifying (●) cells. Measurements were conducted at 15°C, 500 μmol m⁻² s⁻¹ and 2.0 mM Cᵢ (inorganic carbon). (n = 7 ± SD)

Carbonic Anhydrase (Act. Unit)

Fig. 6. *Pleurochrysis* sp. and *P. elongata*. Effect of acetazolamide on the activity of carbonic anhydrase (CA) for low calcifying (○) cells and high calcifying (●) cells. (CA activity is presented relative to a control consisting of 5 mM TRIS-HCl buffer solution or boiled cell suspensions mixed with CO₂-saturated distilled water) (n = 7 ± SD)

Fraction of CA activity by 1.0 mM AZ averaged 74% for LC cells (p < 0.01) and 70% for HC cells (p < 0.01), while at 0.2 mM AZ it averaged 9% (p < 0.05) for LC cells and 58% (p < 0.01) for HC cells (Fig. 6).

The response of photosynthetic O₂ evolution to increasing concentrations of external Cᵢ is depicted in Fig. 7. The CO₂ concentration required for a half-maximal rate of photosynthetic O₂ evolution, Kᵢ₅(CO₂), calculated from double-reciprocal plots of net photosynthesis at pH 5.0 was 37 and 51 μM for LC and HC cells, respectively (Fig. 7A). The Cᵢ concentration required for a half-maximal rate of photosynthetic O₂ evolution, Kᵢ₅(Cᵢ), obtained from double-reciprocal plots of net photosynthesis at pH 7.5 was 0.58 and 0.21 mM for LC and HC cells, respectively (Fig. 7B), while Kᵢ₅(Cᵢ) at pH 8.5 was 1.25 and 0.55 mM for LC and HC cells, respectively (Fig. 7C).

Table 2. *Pleurochrysis* sp. and *P. elongata*. The effect of photosynthetic photon flux (PPF) on rates of calcium uptake for low calcifying (LC) cells and high calcifying (HC) cells. Measurements were conducted at 15°C, pH 7.5 and 2.0 mM Cᵢ (inorganic carbon). (n = 7 ± SD)

<table>
<thead>
<tr>
<th>PPF (μmol m⁻² s⁻¹)</th>
<th>Calcium uptake (nmol Ca²⁺ 10⁶ cells⁻¹ h⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LC</td>
</tr>
<tr>
<td>500</td>
<td>9.2 ± 2.9</td>
</tr>
<tr>
<td>75</td>
<td>10.9 ± 2.1</td>
</tr>
<tr>
<td>Dark</td>
<td>1.2 ± 0.7</td>
</tr>
</tbody>
</table>
Table 3. Non-enzymatic production of CO$_2$ from 100 µM HCO$_3$ dissolved in seawater at 15°C (dCO$_2$/dt; µmol CO$_2$ ml$^{-1}$ h$^{-1}$) and net photosynthetic rates (NPS; µmol O$_2$ ml$^{-1}$ h$^{-1}$) measured with 0.25 mM acetazolamide at various external pH for low calcifying (LC) cells and high calcifying (HC) cells of Pleurochrysis. The first set of calculations of dCO$_2$/dt (Johnson 1982, Eq. 6) assumes that photosynthesizing cells remove all CO$_2$ as rapidly as it is formed from dehydration of HCO$_3^-$, thus CO$_2$ tends to zero. The second set of calculations assumes that cells maintain a CO$_2$ compensation point of approximately 1 µM. (n = 5 ± SD)

<table>
<thead>
<tr>
<th>pH</th>
<th>6.0</th>
<th>7.5</th>
<th>8.0</th>
<th>9.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>dCO$_2$/dt (zero CO$_2$)</td>
<td>1.901</td>
<td>0.064</td>
<td>0.024</td>
<td>0.006</td>
</tr>
<tr>
<td>dCO$_2$/dt (1 µM CO$_2$)</td>
<td>1.883</td>
<td>0.049</td>
<td>0.006</td>
<td>-0.039</td>
</tr>
<tr>
<td>NPS-LC</td>
<td>0.071</td>
<td>0.024</td>
<td>0.017</td>
<td>0.009</td>
</tr>
<tr>
<td>NPS-HC</td>
<td>0.048</td>
<td>0.021</td>
<td>0.025</td>
<td>0.020</td>
</tr>
</tbody>
</table>

Rates of calcium uptake for HC cells averaged 200% higher (p < 0.01) at saturating PPF and 122% higher (p < 0.01) at subsaturating PPF than rates of calcium uptake for LC cells (Table 2). Calcium uptake in the dark was 323% higher (p < 0.01) in HC cells than in LC cells and averaged 13 and 24% of the calcium uptake rates of illuminated LC cells and HC cells, respectively (Table 2).

Comparisons between photosynthetic rates and the CO$_2$ supplied from non-enzymatic dehydration rates of HCO$_3^-$ (100 µM) showed that the latter was sufficient to account for observed photosynthesis of both types of cell at pH 6.0 and pH 7.5 (p < 0.01; Table 3). Relative concentrations of CO$_2$ and HCO$_3^-$ vary with pH, and rates of CO$_2$ formation will depend on the compensation concentration of CO$_2$ maintained by the cells in the experimental setup. At pH 8.0 and assuming zero CO$_2$ utilization rates of CO$_2$ during photosynthesis in both HC and LC cells were within the range of the CO$_2$ being produced spontaneously from HCO$_3^-$ (p > 0.05), while at 1 µM, photosynthetic CO$_2$ uptake was substantially higher than CO$_2$ formation at that pH (p < 0.01; Table 3). At pH 9.0 rates of non-enzymatic CO$_2$ production were not sufficient to support the rates of photosynthesis observed for both LC and HC cells (p < 0.01; Table 3).

**DISCUSSION**

The PPF at which photosynthetic rates of LC and HC cells were maximal exceeded the PPF available to the cultures during cell growth (about 75 µmol m$^{-2}$ s$^{-1}$), and photoinhibition was not observed in either strain at a PPF as high as 1000 µmol m$^{-2}$ s$^{-1}$. The higher initial rates (see Fig. 1) observed for HC cells at low PPF suggest that Pleurochrysis sp. has a decided advantage for growth, even though the content of chlorophyll a was lower than that in LC cells of P. elongata. The range of light intensities tolerated by these strains enables them to withstand a wide range of environmental light conditions; similar light tolerance characteristics have been found for several other microalgal species.

Since the intracellular concentration of oxygen produced during photosynthesis can reach levels far above normal atmospheric levels, the inhibition of photosynthesis observed at high O$_2$ was most likely the result of competition of substrates, CO$_2$ versus O$_2$, for the active site of the carboxylating/oxygenating enzyme, Rubisco. When aliquots of C, were added to the cell suspensions at approximately 280 µM O$_2$, a sharp increase in the photosynthetic O$_2$ evolution was observed. O$_2$ evolution could also be re-established by acidifying the medium with HCl or by reducing O$_2$ levels back to 20 µM thus modifying the CO$_2$:O$_2$ ratios in the measuring chamber.

Inorganic carbon at pH 9.0 is mainly CO$_2^-$ with less HCO$_3^-$ and much less CO$_2$. In particular, a fixed amount of C, at pH 9.0 and 15°C maintained in a closed system will contain about 1% CO$_2$ and 47% HCO$_3^-$, whereas at pH 5.0 about 90% will consist of CO$_2$ and only 6% will be HCO$_3^-$. The photosynthetic activity measured at a range of external pH indicates that HC cells utilize HCO$_3^-$ more efficiently than LC cells, with LC cells relying more exclusively on a CO$_2$ supply. The C, preference shown by Pleurochrysis is similar to that reported for Emiliania huxleyi (Nimer et al. 1992, Nimer & Merrett 1993). Even though both strains of Pleurochrysis benefit from enhanced CO$_2$ availability at low ambient pH, LC cells were still capable of maintaining minimal photosynthesis at alkaline pH where CO$_2$ concentration is low.

The slight alkalinization of the external medium during photosynthesis is, in Pleurochrysis, comparable to that reported for high calcifying cultures of Emiliania huxleyi (Nimer et al. 1992, Dong et al. 1993). These results are also comparable to findings on several microalgae which have CO$_2$-concentrating systems (Shiraiwa et al. 1993) that maintain pH balances intracellularly and generally increase pH extracellularly. However, in the case of Pleurochrysis and probably other coccolithophors, the extent of external alkalinization is reduced because protons are produced as part of the CaCO$_3$ deposition during photosynthesis. This effect reduces alkalinity of the cytosol and favors release of CO$_2$ from HCO$_3^-$ which further increases the availability of substrate for photosynthesis (Raven 1991, McConnaughey 1994). Conversely, the fact that alkalinization of the medium of the LC cells was more pronounced may reflect the lower calcification and
lower production of protons from the acidic reaction. Thus, in the absence of calcification, the consequent alkalization of the cytosol may be countered by expulsion of OH⁻ from the cell or, alternatively, recruitment of H⁺ from the medium.

In contrast to the response found in HC cells of Pleurochrysis sp. at pH 8.5 where photosynthesis reached saturation at 2.2 mM C₅, photosynthesis in LC cells was not carbon saturated at the C₅ levels of seawater (2.1 mM); this is consistent with previous results for air-grown, LC cells of a strain of Emiliania huxleyi (Nimer et al. 1992, Nimer & Merrett 1993). Furthermore, K₀.₅(CO₂) averaged 44 μM for Pleurochrysis reflecting a low affinity for CO₂. This low affinity was comparable to that of a HC strain of E. huxleyi, which had a K₀.₅(CO₂) of 55 μM (Sekino & Shiraiwa 1994). These values are higher than the CO₂ concentration of seawater (ca 15 μM) suggesting HCO₃⁻ utilization to yield CO₂ intra- and/or extracellularly. In another study Nimer & Merrett (1992) reported a K₀.₅(CO₂) (measured by C₅ uptake at pH 5.0) of 115 μM for a HC E. huxleyi and 12.5 μM for a LC strain. Similar affinities for CO₂ have been found for other unicellular algae, e.g. a K₀.₅(CO₂) of 57 μM at pH 8.0 was measured for Phaeodactylum tricornutum (Patel & Merrett 1986) and 50 μM at pH 8.0 for Porphyridium purpureum (Dixon et al. 1987), both having CO₂-concentrating systems but lacking calcification.

The effect of AZ on CA activity in intact cells can be regarded as inhibition of periplasmic CA (Moroney et al. 1985), although there are indications that some internal CA may also be inhibited (Palmqvist et al. 1990). Photosynthesis in Pleurochrysis was inhibited by AZ to a similar extent for both cell types at concentrations an order of magnitude higher than those used in other studies (Palmqvist et al. 1994), but the inhibition was less for LC cells at low AZ concentrations, indicating that for HC cells C₅ fixation relies on extracellular, CA mediated conversions of HCO₃⁻. Fractions of cell homogenates of Pleurochrysis lacked CA activity, except for a chloroplastic CA (Quiroga & González 1993) that may likely act to maintain CO₂ levels at the site of Rubisco (Nimer et al. 1994). Additionally, CA was absent in cell homogenates or intact cells of Emiliania huxleyi (Sekino & Shiraiwa 1994), but significant CA activity appeared in the chloroplast of cells in stationary phase (Nimer et al. 1994).

Results from this study indicate that CO₂ produced from non-enzymatic dehydration of 100 μM HCO₃⁻ greatly exceeded rates of photosynthesis at acidic pH. At pH 8.0 rates of CO₂ production from 100 μM HCO₃⁻ were still greater than rates of photosynthesis for LC cells at a zero CO₂ compensation concentration. At pH 9.0, the dehydration rates approximate, or are lower than, those of photosynthesis, supporting the notion that HCO₃⁻ is the substrate at the plasmalemma. Periplasmic dehydration of HCO₃⁻ is suggested by the presence of CA but whether it is a (non)charged species that is translocated across the membrane, or whether protons are recruited from intra- or extracellular sources, is unknown at this time.

Calcium uptake in Pleurochrysis probably reflects the production of coccoliths but is not a direct measure of calcification. It occurred under conditions of illumination as well as in darkness, as was demonstrated for Pleurochrysis carterae whose rates of calcification were similar in both light and dark conditions (van der Wal et al. 1987). In the present study, light to dark calcification ratios in Pleurochrysis were about 2, below the average (10) for most calcareous algae (McConnaughey 1994). An equimolar ratio of calcification to photosynthesis suggests a push-and-pull mechanism. The unavoidable rise in alkalinity in the cytosol when 1 M HCO₃⁻ is converted to CO₂ is counterbalanced by protons released from 1 M HCO₃⁻ during calcite formation in the calcifying vesicle. This push-and-pull mechanism is of significant advantage where HCO₃⁻ is the dominant form of extracellular C₅ and under conditions where a steep cytosolic to extracellular pH gradient exists (Donald et al. 1993, Nimer & Merrett 1993).

In summary, this study suggests that in Pleurochrysis, CO₂ is the principal substrate for photosynthesis in LC cells, and that HC cells can additionally utilize HCO₃⁻. Thus, the alkaline seawater environment potentially places constraints on the cell in terms of CO₂ availability for photosynthesis. This constraint can be overcome by the HC cells because protons liberated during calcification facilitate the movement of HCO₃⁻ into the cell and release of CO₂ for photosynthesis. Low affinities for CO₂ in both strains require increased internal CO₂ concentrations to support photosynthesis, which could be achieved via a CO₂-concentrating system at the plasmalemma or, more likely, through generation of CO₂ (from HCO₃⁻) in the chloroplast and concurrent production of calcite (during coccolith formation) in the intracellular coccolith vesicle.

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LITERATURE CITED


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