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

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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 $CaCO_3$ encrusted structures (coccoliths). *Pleurochrysis*, and other calcifying algae, are potential players in atmospheric CO_2 cycling and the maintenance of global carbon balances. In *Pleurochrysis* sp. and *P. elongata* (hereafter *Pleurochrysis*), photosynthesis was affected by increasing O_2 (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 O_2, (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 O_2 evolution were significantly higher for both strains at acidic pH (e.g. 5.0, containing about 90% CO_2) than at seawater pH (e.g. 8.0, having about 1% CO_2), while at a basic pH (e.g. 9.0, virtually no CO_2 and about 50% HCO_3^-) 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 HCO_3^- users. By comparing seawater CO_2 concentrations (i.e. 15 µM) with calculated $K_{0.5}(CO_2)$, the CO_2 concentration required for a half-maximal rate of photosynthetic O_2 evolution, for *Pleurochrysis* (51 and 37 µM for HC and LC cells, respectively) it follows that CO_2 must be concentrated intracellularly for effective photosynthesis in both strains. Thus, an adequate CO_2 supply depends on HCO_3^- 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 $CaCO_3$ (coccoliths) that, either partially or entirely, cover the cell in a loosely organized layer. Coccolithophorid algae remove large quantities of atmospheric CO_2 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_i) dissolved in seawater is mostly composed of high levels of HCO_3^- (ca 2100 µM) and low concentrations of CO_2 (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 HCO_3^- as the exogenous C_i source for photosynthesis. Some microalgae can translocate HCO_3^- across the plasmalemma for intra-

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cellular conversion to CO_2 (Dixon et al. 1987), while others convert HCO_3^- to CO_2 extracellularly with CO_2 readily diffusing into the cell (Badger et al. 1980). A few algae rely on diffusive entry or active uptake of CO_2 (Raven 1991, Badger 1985). The C_i is thus concentrated intracellularly, enhancing the levels of CO_2 at the site of fixation via ribulose 1,5-bisphosphate carboxylase/oxygenase (Rubisco). Carbonic anhydrase (CA) is associated with one of these 'biophysical C_i-concentrating mechanisms' since it catalyzes the reversible hydration of CO_2 ($H^+ + HCO_3^- \rightleftharpoons H_2CO_3 \rightleftharpoons H_2O + CO_2$). This enzyme is localized in the chloroplast stroma and/or bound to the plasmalemma (Moroney et al. 1985).

Studies on transport and fixation of C_i 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 CO_2 concentrations of seawater (Sikes et al. 1980, Nimer et al. 1992). Furthermore, it is known that *E. huxleyi* utilizes HCO_3^- for calcification and photosynthesis, although a low calcifying strain has shown poor affinity for HCO_3^- and preferentially uses CO_2 (Nimer & Merrett 1992, Sekino & Shiraiwa 1994).

If bicarbonate is a source of CO_2 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 CO_2 is taken in across the plasmalemma, the acidotic calcification reactions may not be necessary for sustaining photosynthesis. Current models for C_i 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 $HCO_3^- \rightarrow CO_2$ rates (Sikes & Wheeler 1982, Quiroga & González 1993).

The present investigation compares 2 strains of *Pleurochrysis* differing in their calcification capacity. The relationships among C_i 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 C_i 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 O_2 evolution was measured from 4.0 ml cell suspensions (averaging $4.7 \pm 0.8 \times 10^5$ cell ml^{-1}, $n = 5 \pm SD$) with an O_2 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, O_2 in the cell suspension was reduced to desired levels by gentle bubbling with N_2 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 O_2 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 O_2 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 (C_i) in the O_2 electrode chamber Measurements were continued until stable rates of photosynthesis were recorded.

Photosynthesis under carbon limitation. CO_2-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 CO_2-free artificial seawater medium (450 mM NaCl, 10 mM $CaCl_2$, 10 mM KCl and 30 mM $MgSO_4$) for an additional 30 min under illumination. This technique for cell collection was preferred because centrifugation apparently reduced the photosynthetic capacity of *Pleurochrysis*. C_i-dependent O_2 evolution was measured after $NaHCO_3$ (from freshly prepared stock solutions) was added to cells suspended in CO_2-free artificial seawater medium. The bicarbonate-free O_2 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 $NaHCO_3$ stock solution were injected into the electrode chamber to generate a range of C_i 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.

After HCl treatment, pH was adjusted and C_i added. This treatment effectively removes external, coccolith $CaCO_3$.

Changes in the ambient pH were measured directly with a pH electrode in the 3 ml cell suspension contained in the electrode glass chamber. To keep a gas-tight system, the tip of the pH electrode was fitted with a rubber O-ring to seal off the opening of the glass chamber. Next, the light was turned on and changes in pH and O_2 evolution were recorded simultaneously over 20 to 30 min periods. Rates of net photosynthesis were followed as O_2 increased in the chamber from about 2 to 30%. In other experiments, the pH of the medium was decreased to 6.0 with 10 mM MES buffer, when the concentration of O_2 reached 280 µM (25% in air) or, alternatively, the C_i in the medium was increased by additions of 2 mM HCO_3^-; and photosynthetic rates were again recorded.

Carbonic anhydrase. The activity of extracellular CA of intact cells was estimated by measuring the time-course of pH decrease (from 8.0 to 6.5) with a pH meter (Autex 4500, Beckman, Fullerton, CA) connected to a chart-recorder. When the pH of a mixture consisting of 4 ml cell suspension and 1 ml 5 mM TRIS-HCl buffer solution at 4°C had stabilized at 8.2, 0.5 ml CO_2-saturated distilled water was added and the decrease in pH was recorded. Blanks were prepared by mixing 1 ml buffer solution with 4 ml seawater medium, or boiled cell suspensions prior to the addition of the CO_2-saturated distilled water. The effect of acetazolamide (AZ), a non-membrane-permeating inhibitor, on CA was determined. Increasing amounts of the inhibitor were added to the cell-buffer mixture immediately before the hydration reaction was initiated with CO_2.

Calcium uptake. Rates of calcium sequestration by cells of *Pleurochrysis* were determined by means of a colorimetric method (Pethig et al. 1989). Ca^{2+} ions were assayed by a chromogenic calcium chelator, 5,5'-dinitro BAPTA free acid (BAPTA, Molecular Probes, Inc., Eugene, OR), in the cell medium at the beginning and end of timed incubations. A cell suspension (4 ml; containing 9.1 ± 1.2 mM Ca^{2+}, n = 5 ± SD) was incubated at 15°C for 1.5 to 2.0 h (in 5 ml glass chambers open to the atmosphere and occasionally bubbled with N_2 to avoid O_2 build up) at fluxes of 500, 75 µmol m^{-2} s^{-1} or darkness. Then, 1.0 ml of the cell suspension was withdrawn, centrifuged at 13000 × *g* for 3 min and the supernatant mixed with 20 µl of a 1.0 mM BAPTA solution. After 5 min the color was measured at 426 nm in a spectrophotometer (Beckman 25, Beckman). Concentrations were calculated using regression equations derived from $CaCl_2$ calibration curves.

Other methods and calculations. Cell number per ml of culture was determined with a hemocytometer. 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 (dCO_2/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 µmol m^{-2} s^{-1} (Fig. 1). At 50 µmol m^{-2} s^{-1} (i.e. subsaturating PPF) the initial slope of the photosynthetic reaction was steeper and net photosynthesis averaged 34% higher levels for HC cells than for LC cells ($p < 0.01$; Fig. 1). At 500 µmol 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 µmol m^{-2} s^{-1}) the content of chlorophyll *a* was 20% higher in LC cells compared to HC cells (0.47 ± 0.02 µg chl *a* 10^6cells^{-1} vs 0.39 ± 0.03 µg chl *a* 10^6cells^{-1}, n = 8 ± SD).

At ambient levels of O_2 (21% in air corresponding to 227 µM 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). When O_2 concentrations averaging 230 µM

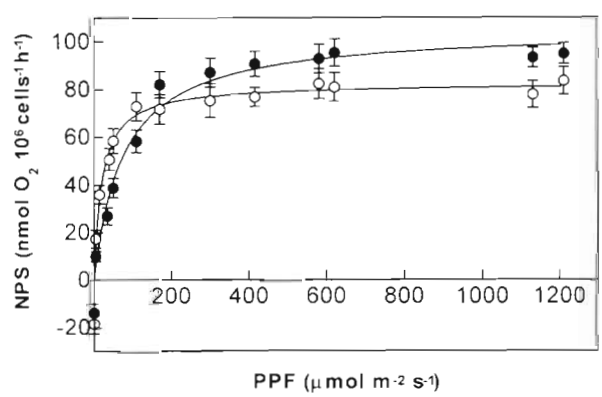

Fig. 1 *Pleurochrysis* sp. and *P. elongata*. Rates of net photosynthesis (NPS) as a function of photosynthetic photon flux (PPF) for low calcifying (●) cells and high-calcifying (○) cells. Measurements were conducted at 15°C, pH 7.5 and 2.0 mM C_i (inorganic carbon). (n = 5 ± SD)

Table 1. *Pleurochrysis* sp. and *P. elongata*. Rates of net photosynthesis (NPS) in response to extracellular O_2 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_i (inorganic carbon). (n = 8 ± SD)

O_2 (µ M)	O_2 in Air (%)	NPS (nmol O_2 10^6 $cell^{-1}$ h^{-1}) LC	HC
14.2–28.4	1.3–2.6	73.6 ± 6.7	65.8 ± 4.9
198.6–255.4	18.4–23.6	60.1 ± 5.8	59.7 ± 4.3
340.5–368.9	31.5–34.1	56.9 ± 4.7	57.4 ± 3.5

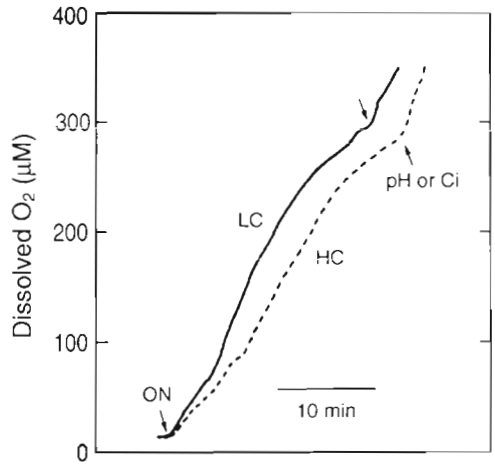

Fig. 2. *Pleurochrysis* sp. and *P. elongata*. Example of an O_2 evolution trace (redrawn) for low calcifying (LC, solid line) and high calcifying (HC, dashed line) cells which were initially bubbled with N_2 + CO_2 to reduce existing O_2 levels to approximately 20 µM, and then illuminated (on) at pH 7.5 and 2.0 mM C_i (inorganic carbon). After O_2 inhibition was apparent, the CO_2:O_2 ratio was increased by adjusting the pH to 6.0 (see Fig. 3), or by increasing the C_i concentration to 2 mM C_i (pH/C_i)

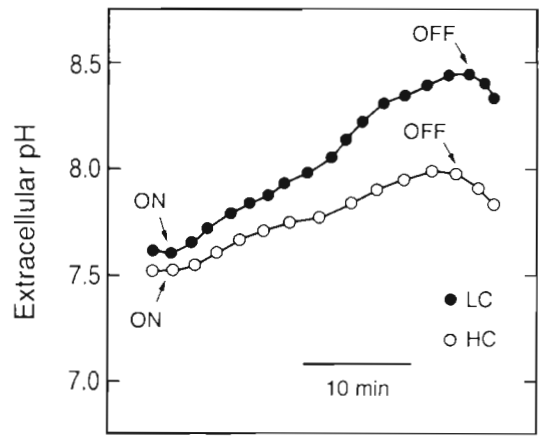

Fig. 3. *Pleurochrysis* sp. and *P. elongata*. Typical experiment showing extracellular pH changes during photosynthesis (on) or darkness (off) for low calcifying (●) cells and high calcifying (○) cells. Measurements were conducted at 15°C, 500 µmol m^{-2} s^{-1} and 2.0 mM C_i (inorganic carbon)

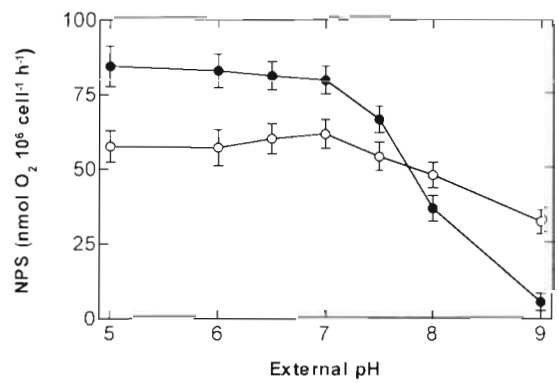

Fig. 4. *Pleurochrysis* sp. and *P. elongata*. Rates of net photosynthesis (NPS) as a function of ambient pH for low calcifying (●) cells and high calcifying (○) cells. Measurements were conducted at 15°C, 500 µmol m^{-2} s^{-1} and 2.0 mM C_i (inorganic carbon) (n = 7 ± SD)

increased to about 350 µM, rates of photosynthetic O_2 evolution decreased, on average, by 23% ($p < 0.01$) for LC cells and by 13% ($p > 0.05$) for HC cells (Table 1).

Photosynthetic O_2 evolution became inhibited as O_2 levels increased within the electrode chamber (Fig. 2, Table 1). For both LC cells and HC cells, rates of photosynthesis at ≥250 µM O_2 could be enhanced to the rates more typically observed at 5% O_2 if the ambient pH was reduced to 6.0, or if C_i 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 O_2 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 O_2 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-

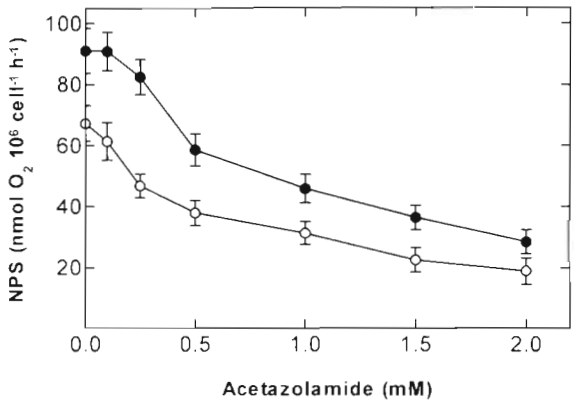

Fig. 5. *Pleurochrysis* sp. and *P. elongata*. Effect of acetazolamide on net photosynthesis (NPS) for low calcifying (●) cells and high calcifying (○) cells. Measurements were conducted at 15°C, 500 µmol m^{-2} s^{-1} and 2.0 mM C_i (inorganic carbon). (n = 7 ± SD)

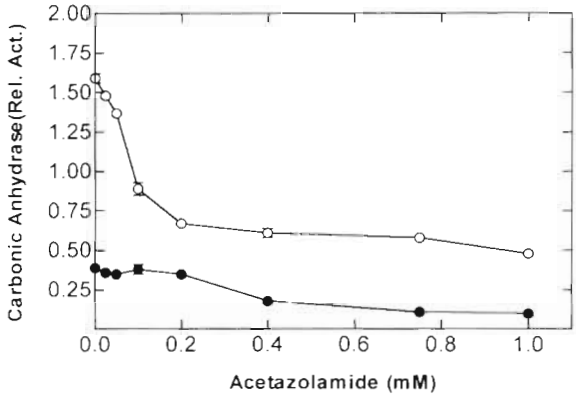

Fig. 6. *Pleurochrysis* sp. and *P. elongata*. Effect of acetazolamide on the activity of carbonic anhydrase (CA) for low calcifying (●) cells and high calcifying (○) cells. (CA activity is presented relative to a control consisting of 5 mM TRIS-HCl buffer solution or boiled cell suspensions mixed with CO_2-saturated distilled water.) (n = 7 ± SD)

tion 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 O_2 evolution to increasing concentrations of external C_i is depicted in Fig. 7. The CO_2 concentration required for a half-maximal rate of photosynthetic O_2 evolution, $K_{0.5}(CO_2)$, 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 C_i concentration required for a half-maximal rate of photosynthetic O_2 evolution, $K_{0.5}(C_i)$, 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 $K_{0.5}(C_i)$ at pH 8.5 was 1.25 and 0.55 mM for LC and HC cells, respectively (Fig. 7C).

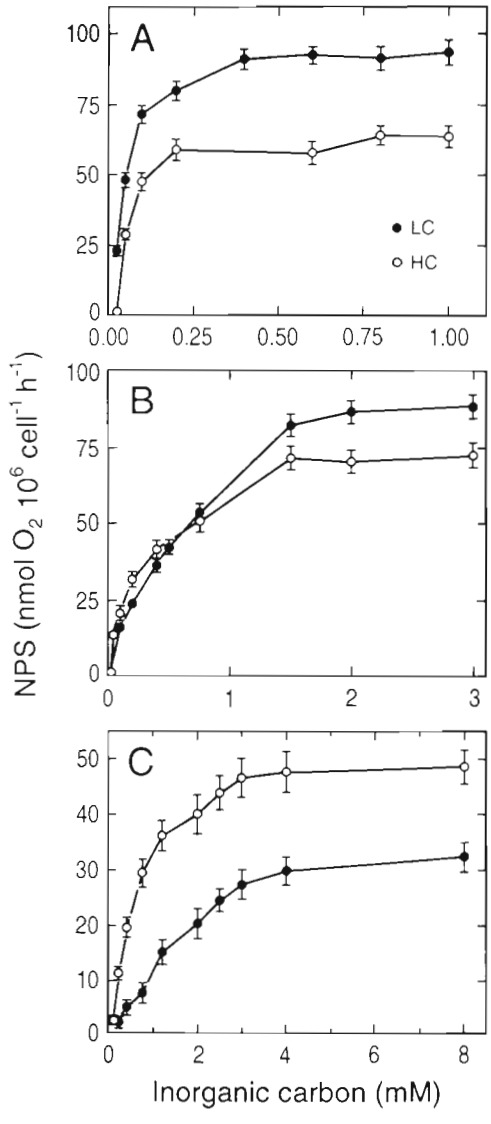

Fig. 7. *Pleurochrysis* sp. and *P. elongata*. Effect of C_i (inorganic carbon) concentration on net photosynthesis (NPS) for low calcifying (●) cells and high calcifying (○) cells at pH 5.5 (A), 7.5 (B), and 8.5 (C). Measurements were conducted at 15°C and 500 µmol m^{-2} s^{-1}. (n = 4 or 5 ± SD)

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 C_i (inorganic carbon). (n = 7 ± SD)

PPF (µmol m^{-2} s^{-1})	Calcium uptake (nmol Ca^{2+} 10^6cells^{-1} h^{-1}) LC	HC
500	9.2 ± 2.9	27.7 ± 3.3
75	10.9 ± 2.1	24.2 ± 3.1
Dark	1.2 ± 0.7	5.1 ± 1.3

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 cell^{-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)

	pH			
	6.0	7.5	8.0	9.0
dCO_2/dt (zero CO_2)	1.901	0.064	0.024	0.006
dCO_2/dt (1 µM CO_2)	1.883	0.049	0.006	−0.039
NPS-LC	0.071	0.014	0.017	0.009
NPS-HC	0.048	0.021	0.025	0.020

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 325% 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_i 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_3^{2-} with less HCO_3^- and much less CO_2. In particular, a fixed amount of C_i 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_i 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 coccolithophorids, 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 acidotic reaction. Thus, in the absence of calcification, the consequent alkalinization 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 C_i, photosynthesis in LC cells was not carbon saturated at the C_i 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, $K_{0.5}(CO_2)$ averaged 44 µM for *Pleurochrysis* reflecting a low affinity for CO_2. This low affinity was comparable to that of a HC strain of *E. huxleyi*, which had a $K_{0.5}(CO_2)$ of 55 µM (Sekino & Shiraiwa 1994). These values are higher than the CO_2 concentration of seawater (ca 15 µM) suggesting HCO_3^- utilization to yield CO_2 intra- and/or extracellularly. In another study Nimer & Merrett (1992) reported a $K_{0.5}(CO_2)$ (measured by C_i uptake at pH 5.0) of 115 µM for a HC *E. huxleyi* and 12.5 µM for a LC strain. Similar affinities for CO_2 have been found for other unicellular algae, e.g. a $K_{0.5}(CO_2)$ of 57 µM at pH 8.0 was measured for *Phaeodactylum tricornutum* (Patel & Merrett 1986) and 50 µM at pH 8.0 for *Porphiridium purpureum* (Dixon et al. 1987), both having CO_2-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 C_i fixation relies on extracellular, CA mediated conversions of HCO_3^-. Fractions of cell homogenates of *Pleurochrysis* lacked CA activity, except for a chloroplastic CA (Quiroga & González 1993) that may likely act to maintain CO_2 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 CO_2 produced from non-enzymatic dehydration of 100 µM HCO_3^- greatly exceeded rates of photosynthesis at acidic pH. At pH 8.0 rates of CO_2 production from 100 µM HCO_3^- were still greater than rates of photosynthesis for LC cells at a zero CO_2 compensation concentration. At pH 9.0, the dehydration rates approximate, or are lower than, those of photosynthesis, supporting the notion that HCO_3^- is the substrate at the plasmalemma. Periplasmic dehydration of HCO_3^- 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 HCO_3^- is converted to CO_2 is counterbalanced by protons released from 1 M HCO_3^- during calcite formation in the calcifying vesicle. This push-and-pull mechanism is of significant advantage where HCO_3^- is the dominant form of extracellular C_i and under conditions where a steep cytosolic to extracellular pH gradient exists (Dong et al. 1993, Nimer & Merrett 1993).

In summary, this study suggests that in *Pleurochrysis*, CO_2 is the principal substrate for photosynthesis in LC cells, and that HC cells can additionally utilize HCO_3^-. Thus, the alkaline seawater environment potentially places constraints on the cell in terms of CO_2 availability for photosynthesis. This constraint can be overcome by the HC cells because protons liberated during calcification facilitate the movement of HCO_3^- into the cell and release of CO_2 for photosynthesis. Low affinities for CO_2 in both strains require increased internal CO_2 concentrations to support photosynthesis, which could be achieved via a CO_2-concentrating system at the plasmalemma or, more likely, through generation of CO_2 (from HCO_3^-) in the chloroplast and concurrent production of calcite (during coccolith formation) in the intracellular coccolith vesicle.

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