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Quantification of the Contribution of CO_2, HCO_3^-, and External Carbonic Anhydrase to Photosynthesis at Low Dissolved Inorganic Carbon in *Chlorella saccharophila*[1]

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An equation has been developed incorporating whole-cell rate constants for CO_2 and HCO_3^- that describes accurately photosynthesis (Phs) in suspensions of unicellular algae at low dissolved inorganic carbon. At pH 8.0 the concentration of CO_2 available to the algal cells depends on the rate of supply from, and the loss to, HCO_3^- and the rate of use by the cells. At elevated cell densities (>30 mg chlorophyll [Chl] L^{-1}), at which CO_2 use by the cells is high, the slope of a graph of absolute Phs versus Chl concentration approaches the rate of Phs on a milligram of Chl basis because of HCO_3^- use alone. The slope of a graph of Phs versus HCO_3^- will be the rate constant for HCO_3^-, and for *Chlorella saccharophila* it was 0.16 L mg^{-1} Chl h^{-1}. The difference between the constants for dissolved inorganic carbon (measured in cells with external carbonic anhydrase) and HCO_3^{-1} is the constant for CO_2, which was 26 L mg^{-1} Chl h^{-1}. This difference causes the half-saturation constant for Phs to increase 5- to 6-fold at high cell densities. The increase in CO_2 use as a result of external carbonic anhydrase is described mathematically as a function of cell density.

The ability to accumulate DIC has been shown to occur in many algae and cyanobacteria (Raven, 1991, and refs. therein). The physiological implications of DIC accumulation include low CO_2-compensation points (Birmingham and Colman, 1979), high whole-cell affinity for DIC (Berry et al., 1976; Kaplan et al., 1980), and suppression of photorespiration (Colman, 1989). The DIC-concentrating mechanism is thought to consist of HCO_3^- and/or CO_2 transport as distinct processes (Miller and Canvin, 1985; Thielmann et al., 1990).

In some green algae the use of HCO_3^- has been correlated with the presence of external CA activity (Miyachi et al., 1983; Moroney et al., 1985; Aizawa and Miyachi, 1986; Nara et al., 1990). In these cases external CA is thought to facilitate the use of HCO_3^- by maintaining equilibrium between HCO_3^- and CO_2 and thereby maintaining the supply of CO_2 to a CO_2 transporter (Aizawa and Miyachi, 1986). Direct HCO_3^- transport, however, has also been demonstrated in cells that have external CA activity (Williams and Turpin, 1987; Gehl et al., 1990). External CA could increase overall DIC supply at alkaline pH if CO_2 is the preferred species of DIC as has been shown in some green algae (Shelp and Canvin, 1980; Sültemeyer et al., 1989). The precise contribution of external CA, HCO_3^- transport, and CO_2 transport to Phs has yet to be elaborated.

In the unicellular green alga *Chlorella saccharophila* the accumulation mechanism includes both CO_2 (Rotatore and Colman, 1991) and HCO_3^- (Gehl et al., 1990) transporters. *C. saccharophila* also has an external form of CA (Williams and Colman, 1993) that is suppressed by growth at acid pH (Gehl et al., 1990). Transport of HCO_3^- in *C. saccharophila* has been shown using the kinetic method of Miller and Colman (1980). This method involves showing that in the absence of external CA activity a dense culture of cells photosynthesizes more rapidly than the theoretical maximum spontaneous production of CO_2 from HCO_3^-. Phs above that of the CO_2 production rate is presumably the result of HCO_3^- transport. This method, although yielding strong evidence for the presence of HCO_3^- transport, does not quantify how much direct HCO_3^- transport is contributing to Phs. The isotopic disequilibrium method described by Espie and Colman (1986) is powerful at discerning HCO_3^- and CO_2 use but does so at elevated DIC levels and is quantitative only if a single species of DIC is used by an organism.

The transport of CO_2 in *C. saccharophila* has been demonstrated using MS (Rotatore and Colman, 1991). The CO_2 concentration in a suspension of cells was monitored after illumination, and it was shown that acid-grown cells of *C. saccharophila* were able to remove CO_2 to approximately a zero concentration within 2 min. The inorganic carbon species were maintained out of equilibrium as shown by the increase in CO_2 upon addition of exogenous CA.

Both of these methods use the fact that the uncatalyzed interconversion of HCO_3^- and CO_2 is slow relative to photosynthetic requirements for CO_2. These ideas can be extended to an extreme case in which the demand for CO_2 is far greater than the spontaneous supply rate, at which point the cells would photosynthesize as a function of the supply of HCO_3^- alone. At very high cell-density CO_2 supply to total Phs will approach a constant rate at or near the theoretical maximum supply rate from HCO_3^-. This allows the examination of the contributions of HCO_3^- and CO_2 to Phs and changes in whole-cell affinity for DIC as Phs becomes dependent on HCO_3^-.

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Abbreviations: CA, carbonic anhydrase; DIC, dissolved inorganic carbon; Phs, photosynthesis; P_{max}, maximum photosynthetic rate.

THEORETICAL CONSIDERATION

If Phs at low DIC is a function of transport of CO_2 and HCO_3^- and these processes are independent and are reasonably described by a linear relationship, then a simple equation can be derived to describe Phs at low DIC.

$$\text{Phs} = k_1[CO_2] + k_2[HCO_3^-] \quad (1)$$

where Phs is Phs as μmol O_2 mg^{-1} Chl h^{-1} assuming a photosynthetic quotient of unity; k_1 is the whole-cell rate constant for Phs due to CO_2 as L mg^{-1} Chl h^{-1}; k_2 is the whole-cell rate constant for Phs due to HCO_3^- as L mg^{-1} Chl h^{-1}; $[CO_2]$ is the CO_2 concentration outside the cell; and $[HCO_3^-]$ is the HCO_3^- concentration outside the cell.

If Phs is given as the absolute rate of O_2 evolution, then the equation becomes

$$\text{Phs}_{abs} = k_1[Chl][CO_2] + k_2[Chl][HCO_3^-] \quad (2)$$

where Phs_{abs} is O_2 evolution as μM O_2 h^{-1} and [Chl] is Chl concentration as mg Chl L^{-1}.

If DIC is added to a suspension of cells at alkaline pH (8.0) as HCO_3^-, then the CO_2 concentration will be a function of the supply of CO_2 from HCO_3^-, its loss in the backreaction to HCO_3^-, and its use by the cells. Let us assume that a steady state between and CO_2 supply occurs much more rapidly than total DIC is used through Phs. Since at pH 8.0 98% of DIC is in the form of HCO_3^-, Phs as a function of HCO_3^- may remain written as stated in Equation 2. CO_2 will be supplied from two reactions involving HCO_3^-. The first is

$$HCO_3^- + H^+ \rightleftharpoons H_2CO_3 \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} H_2O + CO_2 \quad (3)$$

The initial part of this reaction occurs almost instantaneously and so the concentration of H_2CO_3 may be written as a function of the equilibrium constant for the dissociation of H_2CO_3.

$$[H_2CO_3] = \frac{[HCO_3^-][H^+]}{K_{eq}} \quad (4)$$

The other reaction involved in the supply of CO_2 from HCO_3^- is

$$HCO_3^- \underset{k_{-4}}{\overset{k_4}{\rightleftharpoons}} OH^- + CO_2 \quad (5)$$

The loss of CO_2 through the backreactions can be easily described by rate equations. The loss of CO_2 as a result of Phs is a function of the whole-cell affinity for CO_2 and the number of cells present as described by Chl concentration (see Eq. 2). The concentration of CO_2 at steady state thus becomes

$$[CO_2] = \frac{k_4[HCO_3^-] + k_3[H_2CO_3]}{[Chl]k_1 + k_{-3} + k_{-4}[OH^-]} \quad (6)$$

Substituting using Equation 4 we obtain

$$[CO_2] = \frac{[HCO_3^-]k_4 + \dfrac{k_3[HCO_3^-][H^+]}{K_{eq}}}{[Chl]k_1 + k_{-3} + k_{-4}[OH^-]} \quad (7)$$

By rearranging and collecting constants at pH 8.0 we obtain

$$[CO_2] = \frac{k_f[HCO_3^-]}{k_1[Chl] + k_b} \quad (8)$$

where $k_f = k_4 + k_3\dfrac{10^{-8}}{K_{eq}}$ and $k_b = k_{-3} + k_{-4}10^{-6}$.

By substituting Equation 8 into Equation 2, the absolute rate of O_2 evolution becomes

$$\text{Phs}_{abs} = \frac{k_f[HCO_3^-]}{k_1[Chl] + k_b}[Chl]k_1 + k_2[HCO_3^-][Chl] \quad (9)$$

The contribution of CO_2 to Phs takes the form of a rectangular hyperbola when DIC (HCO_3^-) is kept constant and cell density is changed (Fig. 1). As $[Chl]k_1$ becomes large relative to the rate of loss of CO_2 in the backreactions (represented by k_b), then the contribution of CO_2 to Phs approaches an asymptote defined by the maximum rate of CO_2 supply from HCO_3^- as described by Miller and Colman (1980). With the addition of the contribution of HCO_3^- a line describing total O_2 evolution as a function of [Chl] is obtained (Fig. 1).

The presence of external CA activity will increase the P_{max} in a manner dependent on [Chl]. External CA, at a concentration sufficient to establish equilibrium, would

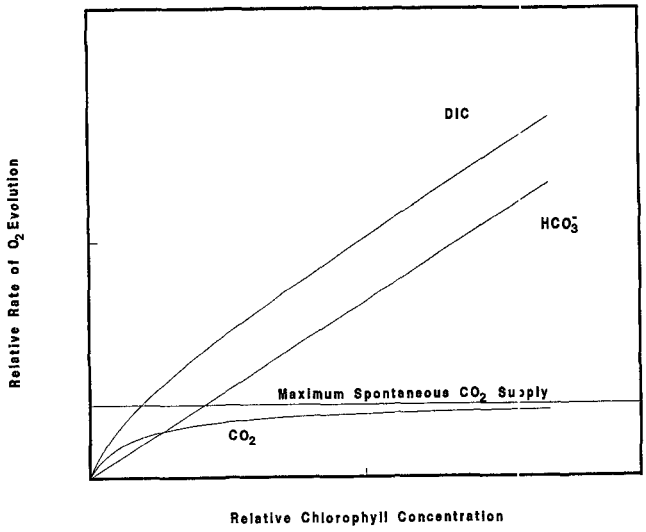

Figure 1. Relative contributions of CO_2 and HCO_3^- to Phs_{abs} as a function of [Chl] as given in Equation 9. As [Chl] increases, DIC is drawn farther out of equilibrium and $[CO_2]$ will approach zero. The contribution of CO_2 thus approaches the maximum dehydration rate of HCO_3^-. The contribution of HCO_3^- is [Chl] independent and so Phs_{abs} will increase in a linear manner with [Chl]. The addition of the two lines gives Phs_{abs} as a function of [Chl] for total [DIC].

maintain the contribution of CO_2 to Phs at

$$\frac{k_f}{k_b}[DIC]k_1[Chl] \quad (10)$$

where the ratio k_f/k_b represents the equilibrium proportion of CO_2 at pH 8.0. A small proportion of DIC will be present as CO_3 at pH 8.0 (approximately 0.5%), but this will have little effect on the model. Since the supply of CO_2 in the absence of external CA activity is defined by Equation 9, then CA will increase use of CO_2 by a factor of

$$\frac{k_1[Chl])}{k_b} + 1 \quad (11)$$

Values for the rate constants for Equations 3, 4, and 5 were obtained from Miller and Colman (1980). It must be stated that the temperature used in these experiments was 22°C, which is 3°C lower than the temperature at which the rate constants were determined. This difference was deemed insignificant in comparison with variability in the reported values for these constants (Pocker and Bjorkquist, 1977; Stumm and Morgan, 1981). When these constants are used to determine theoretical rates of reactions, they must be used with the knowledge that they are simply good approximations. Given this, the values for k_f and k_b were determined to be 4.5 h^{-1} and 164 h^{-1}, respectively.

Values for k_1 and k_2 can be derived experimentally. The slope of the line described by Equation 9 when [DIC] is held constant and as [Chl] becomes large will approach the P_{max} on a milligrams of Chl basis as a result of HCO_3^- alone. By creating a number of graphs at different [HCO_3^-] values, we can create a Phs versus [HCO_3^-] graph, the initial slope of which will be k_2.

When external CA is added or the cells are grown at alkaline pH so that they exhibit external CA activity, the use of CO_2 will become independent of [Chl] and the slope of a graph of Phs versus [DIC] will yield a rate constant for total DIC at equilibrium. Since

$$k_{DIC} = k_1 + k_2 \quad (12)$$

where k_{DIC} is whole-cell rate constant of Phs due to total DIC, then k_1 can be be obtained by difference.

MATERIALS AND METHODS

Chlorella saccharophila (UTCC 91, UTEX 2496) was obtained from the culture collection at the University of Toronto and maintained on Bold's basal medium (Gehl et al., 1990). Cells were grown at pH 8.0 buffered with 10 mM Bicine and at pH 5.0 in semicontinuous culture with the pH maintained by a pH-stat system (Cole-Parmer Co., Chicago, IL). The latter was necessary because the biological buffer Mes was found to suppress CA activity independently of pH and was, therefore, avoided in case other processes were are also affected. Bicine had no effect on external CA activity when compared to cells grown at alkaline pH in the pH-stat system.

CA activity was measured using the potentiometric technique of Wilbur and Anderson (1948) as modified by Williams and Colman (1993). Approximately 30 mL of cell culture were centrifuged, washed once with 10 mL of barbital buffer (pH 8.3), and resuspended in a final volume of either 3.0 or 1.5 mL. CA assays were performed on 1.5-mL aliquots of either 30 mg Chl L^{-1} or 60 mg Chl L^{-1} final concentration. The higher concentrations were used for acid-grown cells to ensure that no CA activity was measurable. Chl concentration was measured as described previously (Williams and Colman, 1993).

O_2 evolution was monitored using a Clarke-type electrode (Hansatech Inc., Norfolk, UK). Cells were centrifuged and washed once with distilled water before being resuspended in low-DIC 25 mM Bicine buffer (pH 8.0) in fresh Bold's medium. Low-DIC buffer was obtained by boiling the buffer while bubbling with N_2 for 1 h. Volume was maintained by the addition of distilled water. Aliquots (1 mL) of cell suspension were placed in the electrode chamber, and the cells were allowed to reach a point at or near to the CO_2-compensation point, at which time the total DIC was measured (approximately 5–10 μM total DIC in both acid- and alkaline-grown cells) using the gas chromatographic technique of Birmingham and Colman (1979). O_2 evolution was then monitored upon incremental addition of $NaHCO_3$. DIC used by the cells was accounted for by assuming a photosynthetic quotient of unity and then subtracting the used DIC from the total added. Phs was determined after a steady rate of O_2 evolution was observed (minimum time approximately 30 s). Chart speeds of up to 15 cm min^{-1} were required at the elevated Chl concentrations. The photosynthetic kinetics were also monitored in acid-grown cells with the addition of 100 μg mL^{-1} bovine CA (Sigma). Illumination was provided by two Fiber-Lite high-intensity illuminators (Dolan-Jenner Industries, Inc., Woburn, MA) equipped with two fiber-optic cables each. The maximum intensity supplied by each cable was approximately 2800 μE m^{-2} s^{-1}. Intensity was varied to avoid light limitation or photoinhibition.

Since rates of DIC-dependent Phs were not measured at exactly the same DIC concentrations at each cell density, comparisons were made between Phs values predicted by fitting the empirical data obtained at low DIC (those below and just above the level yielding half-maximal Phs) to a rectangular hyperbola using a double-reciprocal plot. The actual concentration of DIC that yielded half-maximal Phs ($K_{½}$DIC) was determined mathematically using the derived equations after measuring P_{max} empirically.

RESULTS

Acid-grown cells exhibited no detectable CA activity when assayed at 60 μg Chl mL^{-1}, whereas cells grown at pH 8.0 had 40 units mg^{-1} Chl. To examine changes in DIC-limited Phs with changing cell density, it was first necessary to show that light did not become limiting at elevated densities. This was established by showing that maximum P_{max} did not change over the range of Chl concentrations used with either alkaline-grown cells or acid-grown cells (Fig. 2). The P_{max} for alkaline-grown cells was lower than that of acid-grown cells, probably as a result of a slower growth rate.

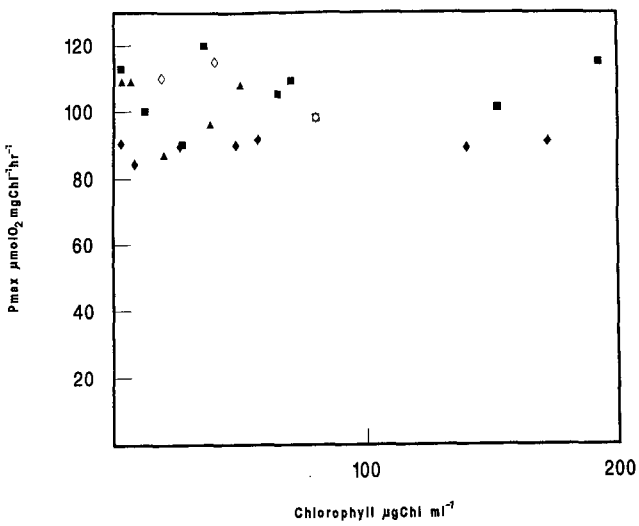

Figure 2. Variation in P_{max} in alkaline-grown cells (♦), acid-grown cells (■, □, ▲), and acid-grown cells with the addition of 100 μg CA mL^{-1} (◇) as a function of cell density. The different symbols within the acid treatment represent results from different experiments.

Absolute rates of O_2 evolution as a function of Chl concentration are shown in Figure 3 for acid-grown cells, alkaline-grown cells, and acid-grown cells with the addition of bovine CA for a single DIC concentration of 50 μM. The slope through all points associated with Chl concentrations greater than 36 mg L^{-1} is shown for acid-grown cells and was 8.07 μmol O_2 mg^{-1} Chl h^{-1}. The regression through points associated with alkaline-grown cells yielded a slope of 30 μmol O_2 mg^{-1} Chl h^{-1}. The addition of external bovine CA to acid-grown cells shifted the Phs values of acid-grown cells to that of alkaline-grown cells (Fig. 3).

The rates of Phs due to HCO_3^- were calculated at a number of DIC concentrations as described in Figure 3. Rates of Phs due to HCO_3^- alone as a function of [DIC] are shown in Figure 4. The slope of this line (k_2 as given in Eq. 1), as determined by regression, is 0.16 L mg^{-1} Chl h^{-1} assuming a photosynthetic quotient of 1. The initial slope of the Phs versus DIC regression was 0.74 L mg^{-1} Chl h^{-1}, giving (by difference) a slope for CO_2 of 0.58 L mg^{-1} Chl h^{-1}. If we assume equilibrium conditions in the presence of external CA activity where the CO_2 is 2.2% of total DIC, the slope for CO_2 in terms of CO_2 was 26 L mg^{-1} Chl h^{-1} (k_1 as given in Eq. 1). The affinity for CO_2 in *C. saccharophila* is, therefore, 160 times higher than that for HCO_3^-.

The graph of Phs_{abs} versus [Chl] as created by Equation 9 for a single [DIC] of 50 μM is plotted with the measured rates of O_2 evolution in Figure 5. Phs generally is described as μmol O_2 mg^{-1} Chl^{-1} h^{-1}. A three-dimensional graph of Phs on a milligram of Chl basis versus [DIC] and [Chl] can be produced from Equation 9 and by subsequently dividing the Phs_{abs} by [Chl] (Fig. 6). This graph represents the initial portion of Phs versus DIC curves at varying Chl concentration. Phs increases linearly with DIC, and the slope of this function increases as Chl concentration (cell density) decreases.

Whereas the P_{max} did not change with increasing cell density the $K_{½}$DIC increased by a factor of 6 in acid-grown cells and increased only slightly in alkaline-grown cells (Fig. 7).

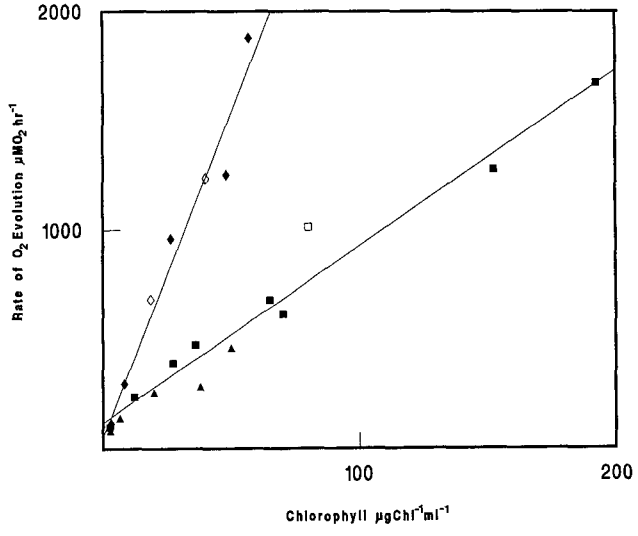

Figure 3. Total O_2 production in alkaline-grown cells (♦), acid grown cells (■, □, ▲), and acid-grown cells with 100 μg CA mL^{-1} (◇) as a function of Chl concentration (a measure of cell density) at 50 μM DIC. The different symbols for acid-grown cells represent results from different experiments. The line drawn through points associated with acid-grown cells between 36 and 192 μg Chl mL^{-1} was determined by linear regression, as was the line drawn for alkaline-grown cells.

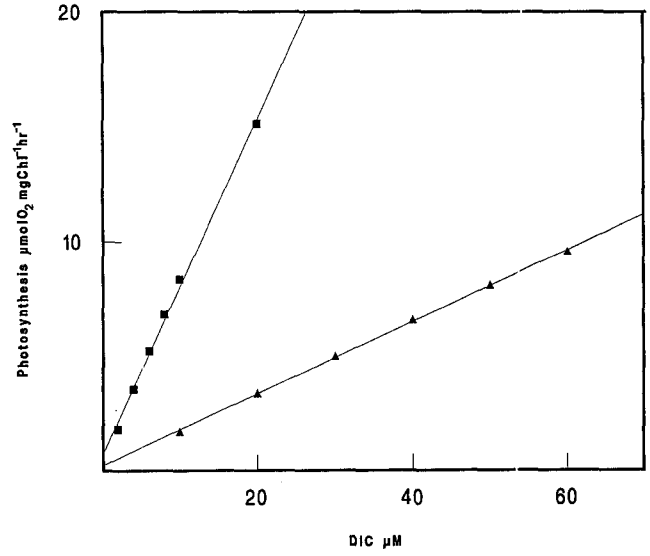

Figure 4. Phs as a function of DIC concentration as a result of HCO_3^- use (▲; from the slopes of Fig. 3 at different [DIC] for acid-grown cells) and DIC (■; from the slopes of Fig. 3 for alkaline-grown cells). The r^2 values for all slopes as calculated by linear regression were all greater than 0.93.

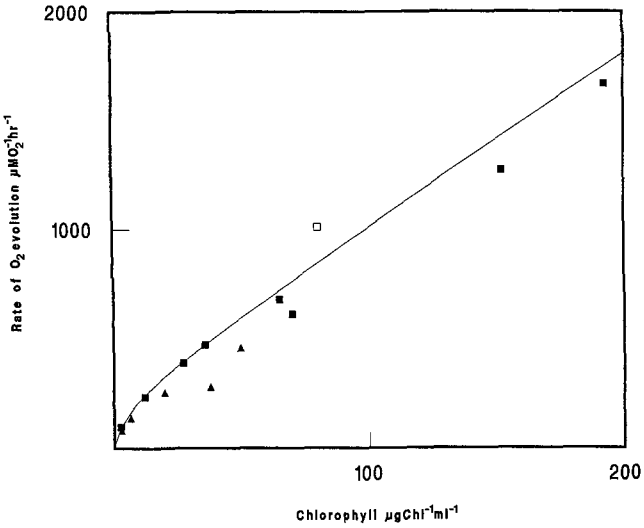

Figure 5. Phs as a function of Chl concentration for cells grown at acid pH (■, □, ▲) at 50 μM DIC. The different symbols represent results from different experiments. The line drawn was created by using the values of k_1 and k_2 calculated from Figure 4 in Equation 9 at 50 μM DIC.

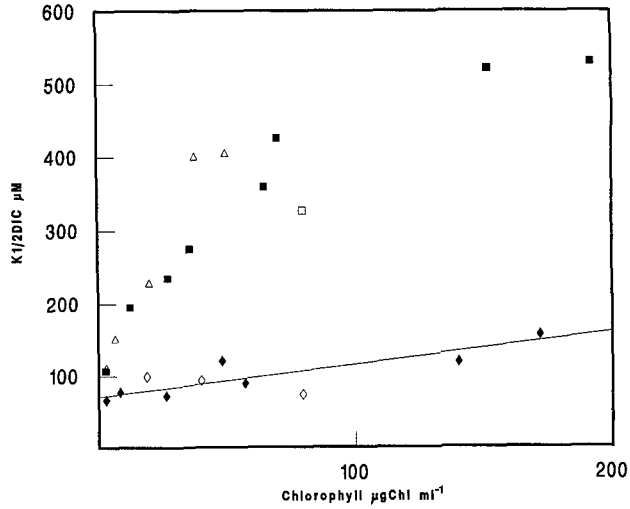

Figure 7. Variation in $K_{½}$DIC in alkaline-grown cells (♦), acid-grown cells (■, □, △), and acid-grown cells with the addition of 100 μg CA mL^{-1} (◇) as a function of cell density. The different symbols within the acid treatment represent results from different experiments.

DISCUSSION

From previous work it is known that *C. saccharophila* has the ability to use CO_2 (Rotatore and Colman, 1991) and HCO_3^- (Gehl et al., 1990) as substrates for Phs. HCO_3^- is transported directly into the cell and will also act to supply the CO_2 transporter at a rate determined by the rate of chemical reactions and by the degree of catalysis due to the presence of CA.

C. saccharophila cells grown at acid pH exhibit no external CA activity (Gehl et al., 1990; Williams and Colman, 1993). Previous studies of CO_2 and HCO_3^- use have relied on inhibition of external CA using CA inhibitors (Moroney et al., 1985; Williams and Turpin, 1987; Gehl et al., 1990). Although these experiments have enabled conclusions to be drawn regarding the presence of HCO_3^- transport, it is difficult to interpret the effects of CA inhibitors on whole-cell Phs since these compounds may inhibit internal CA activity (Williams and Turpin, 1987), PSII activity (Swader and Jacobsen, 1972), or transport activity (Price and Badger, 1989). The use of acid-grown *C. saccharophila* enables the examination of Phs and the role of external CA without the use of inhibitors. The underlying assumption is that fully activated air-grown cells will have the same capacities for CO_2 and HCO_3^- use regardless of the pH at which they were grown. Since the addition of external bovine CA to acid-grown cells alters the photosynthetic kinetics of acid cells to that of alkaline cells (Fig. 7), it is probable that the major, if not only, difference between acid cells and alkaline cells in relation to their abilities to use DIC is the presence of external CA.

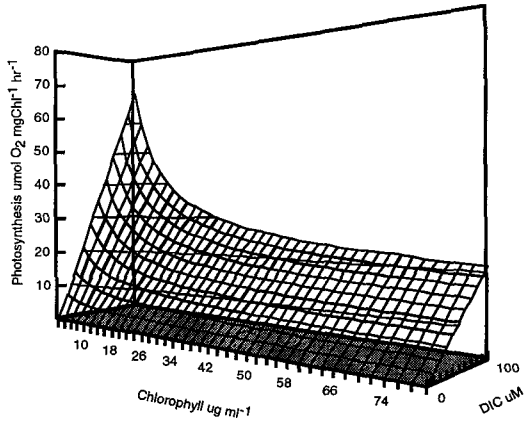

Figure 6. Phs as a function of cell density ([Chl]) and DIC concentration as calculated using Equation 9 and standardized to [Chl]. The constants k_1 and k_2 were calculated from Figure 4.

The precise nature of the use of CO_2 and HCO_3^- in the absence of external CA activity can be described mathematically (Eq. 9) assuming a linear relationship between DIC and Phs at low DIC and that the transporters of HCO_3^- and CO_2 act independently. Previous work has shown that, in a cyanobacterium, the shape of the Phs versus DIC function at low DIC is governed by transport processes and is linear (Mayo et al., 1989). The curved nature of the function at DIC concentrations above half-saturation is a result of limitation of CO_2 fixation by ribulose-1,5-bisphosphate levels (Mayo et al., 1989). This type of result was also seen in the Phs versus DIC functions in this study, wherein the rectangular hyperbolas fitted to the data at DIC concentrations below and just above that required for half-saturation of Phs consistently predicted P_{max} values far in excess of the measured P_{max} data (data not shown). Therefore, Phs is described well by a linear relationship at [DIC] values below the $K_{½}$DIC. However, as [Chl] becomes low, the concentration of DIC required to violate the assumption of linearity will also decrease so that the model will overestimate Phs at [DIC]s of 70 to 100 μM when DIC is at equilibrium (i.e. when [Chl] is very low, see Fig. 6).

The transport of CO_2 and HCO_3^- in a green alga (Thielmann et al., 1989) and a cyanobacterium (Miller and Canvin, 1985) have characteristics suggesting that they are independent processes. Price and Badger (1989) suggested that in a cyanobacterium the transport of HCO_3^- may be through the mediation of a subunit attached to the CO_2 transporter. If this were the case, there could be a control mechanism whereby the HCO_3^- transport is stopped when CO_2 is readily available. The availability of CO_2 is a function of both the $[HCO_3^-]$ and the [Chl] as described in Equation 6. The model described by Equation 9 fits the data of the present study quite well (Fig. 5), suggesting that the assumption may be valid. It is arguable, however, as to whether this method could detect the suppression of HCO_3^- transport at very low cell density given that the percentage contribution of HCO_3^- to Phs is low when CO_2 availability is high.

The estimates of the contribution of HCO_3^- alone to Phs (Fig. 4) will only be accurate if the contribution of CO_2 has become constant and, therefore, close to the maximum spontaneous supply of CO_2 from HCO_3^-. This will only be the case if the CO_2 concentration is approximately zero. This has been shown to be true in acid-grown cells of *C. saccharophila* in which a suspension of cells having a cell density of 8 mg Chl L^{-1} removed CO_2 to about 0.45 μM in a suspension of 100 μM DIC at pH 7.5. The data for Figure 4 were derived from the slopes of Figure 3 at concentrations greater than 36 $\mu g\ mL^{-1}$ and so the CO_2 supply rate is certainly very close to the maximum rate. The rate constant for whole-cell use of HCO_3^- (0.16 L mg $Chl^{-1}\ h^{-1}$) is probably accurate. The estimate for the rate constant for CO_2 (26 L mg^{-1} Chl h^{-1}) may be less accurate because there was a small density effect on Phs independent of CO_2 and HCO_3^- supply (Fig. 7). The values of k_{DIC} and consequently k_1 would therefore be underestimated. Even given these limitations, other data of inorganic carbon fluxes versus $[CO_2]$ observed by Palmqvist et al. (1994) using MS for *Chlamydomonas* and *Scendesmus* would suggest that the estimate of k_1 is reasonable. Since these workers were observing inorganic carbon fluxes as opposed to O_2 evolution in this study, it is clear that, although there may be other factors involved, Phs at low DIC is largely a function of the ability of transporters to maintain supply of inorganic carbon to the cell, as has been clearly shown in a cyanobacterium (Mayo et al., 1989).

Given that the P_{max} did not change with cell density (Fig. 2), it is evident from Figure 6 that, as cell density decreases and the initial slope of the Phs versus [DIC] curve increases, there should be a concomitant decrease in the $K_{½}$DIC even if there is some deviation from the assumption of linearity. This is evident in Figure 7, which shows that the $K_{½}$DIC increases 5 to 6 times as cell density increases. The $K_{½}$DIC for cells with external CA was about 150 μM at high cell density and approximately 500 μM in cells without CA. When expressed in terms of CO_2 the $K_{½}$DIC for cells with CA was 3.3 μM, which is 150 times lower than the $K_{½}$DIC for cells without CA. The decrease in affinity for DIC in cells without external CA at high cell density, therefore, is accounted for by the fact that they are reliant on HCO_3^-, for which they have an affinity that is 160 times lower than for CO_2. In light of the fact that $K_{½}$DIC values are strongly dependent on cell density, especially between 0 and 50 mg Chl L^{-1} (Fig. 7), concentrations at which most studies have been performed, it is necessary to evaluate past data with the knowledge of the cell density at which the experiments were performed.

The presence of external CA activity in alkaline cells allows the cells to maintain DIC at equilibrium (Rotatore and Colman, 1991). As mentioned by Tsuzuki and Miyachi (1991), the effects of adding CA to whole cells will only be seen at higher cell densities. The present model allows the quantification of this effect. By maintaining equilibrium, external CA activity will increase CO_2 use as given in Equation 11. For *C. saccharophila* CA increases CO_2 use by a factor of 0.16 [Chl] + 1 at low DIC. As [Chl] approaches 0, the DIC will approach equilibrium and CA will have an increasingly small impact on the rate of Phs. The model's applicability at very low [Chl] may be weak, since it is unlikely, even around a single cell, that DIC is at perfect equilibrium. The degree of disequilibrium around a single cell will begin to depend on diffusional resistance to CO_2, especially as a function of boundary layer thickness. Although the model may or may not hold at low [Chl], it is clear that more dilute cultures will have a much lower requirement for CA to increase affinity for DIC. The presence and level of external CA in microalgae may well depend not only on the CO_2 concentration in the medium as controlled by CO_2 concentration in the air stream bubbling through a culture but on the density of cells in suspension.

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