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A Model for HCO_3^- Accumulation and Photosynthesis in the Cyanobacterium *Synechococcus* sp.

THEORETICAL PREDICTIONS AND EXPERIMENTAL OBSERVATIONS

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ABSTRACT

A simple model based on HCO_3^- transport has been developed to relate photosynthesis and inorganic carbon fluxes for the marine cyanobacterium, *Synechococcus* sp. Nägeli (strain RRIMP N1). Predicted relationships between inorganic carbon transport, CO_2 fixation, internal carbonic anhydrase activity, and leakage of CO_2 out of the cell, allow comparisons to be made with experimentally obtained data. Measurements of inorganic carbon fluxes and internal inorganic carbon pool sizes in these cells were made by monitoring time-courses of CO_2 changes (using a mass spectrometer) during light/dark transients. At just saturating CO_2 conditions, total inorganic carbon transport did not exceed net CO_2 fixation by more than 30%. This indicates CO_2 leakage similar to that estimated for C_4 plants.

For this leakage rate, the model predicts the cell would need a conductance to CO_2 of around 10^{-5} centimeters per second. This is similar to estimates made for the same cells using inorganic carbon pool sizes and CO_2 efflux measurements. The model predicts that carbonic anhydrase is necessary internally to allow a sufficiently fast rate of CO_2 production to prevent a large accumulation of HCO_3^-. Intact cells show light stimulated carbonic anhydrase activity when assayed using ^{18}O-labeled CO_2 techniques. This is also supported by low but detectable levels of carbonic anhydrase activity in cell extracts, sufficient to meet the requirements of the model.

Photosynthesis and C_i[1] usage in cyanobacteria has been shown to function through the combined operation of RuBP carboxylase activity and a CO_2 concentrating mechanism (7, 9). At alkaline pH values, favored by most cyanobacteria, it is apparent that the bicarbonate ion is the major source of C_i for the concentrating mechanism (2, 10) although it appears that both CO_2 and HCO_3^- may be able to act as substrate for this accumulation (2). The mechanism responsible for C_i uptake is unclear but it is most frequently suggested to involve a HCO_3^- transport system, possibly a primary electrogenic pump (8).

Gas exchange measurements with *Synechococcus* sp. have shown that general characteristics of CO_2 uptake and efflux are consistent with a model of photosynthesis in which HCO_3^- is accumulated within the cell (2). During steady state photosynthesis, CO_2 is constantly effluxing from the cell, being derived presumably from a concentrated internal C_i pool. It is unclear, however, with *Synechococcus* and other cyanobacteria, what the balance is between transport of C_i into the cell, CO_2-fixation, and the leakiness of the cell to CO_2 efflux. The role of CA in the operation of the CO_2 concentrating mechanism is also uncertain.

An attempt is made here to develop a simple quantitative model of photosynthesis in *Synechococcus* sp., deriving relationships between C_i carbon transport, CO_2-fixation, leakage to CO_2, and CA activity. This model is then compared with experimentally obtained estimates of these parameters in air-adapted cells, with good correlation being found.

MATERIALS AND METHODS

The unicellular marine cyanobacterium used in these studies is classified according to Rippka *et al.* (12) as *Synechococcus* sp. Nägeli (strain RRIMP N1) and is an oval rod of dimensions 1.5 × 3 μm. It is considered to belong to the same group as *Agmenellum quadruplication* and *Coccochloris elabens* (11). [^{3}H]inulin and ^3H_2O were obtained from Amersham (UK). [^{18}O]H_2O was from Norsk Hydro (Oslo, Norway).

Growth of *Synechococcus*. The cells were grown to late log phase in 300-ml batches contained in conical flasks. Growth medium was a 0.2 μm filter-sterilized seawater medium based on the f medium of Guillard and Ryther (5), and buffered at pH 8.2 with 50 mM Bicine. Cultures were shaken in a temperature-controlled water bath (30°C) and bubbled with humidified air. Light (400 μmol m^{-2} s^{-1} photon flux density, at the surface of the culture vessels) was provided by a Hg vapor lamp suspended over the bath.

Preparation and Assay of Cells. Cells were harvested by centrifugation at 5000*g* for 10 min, and resuspended in 2 to 3 ml of CO_2-free culture media. The cells were stored in the dark on the bench and slowly bubbled with CO_2-free air prior to their use in all experiments. All assays were performed in seawater medium at pH 8.2 and 30°C.

Cell Parameter Measurements. The internal volume per cell was estimated from silicone oil centrifugation experiments, measuring the [^{3}H]inulin impermeable space of the cells (see Kaplan *et al.* [7]). This was combined with haemocytometer estimates of cell numbers to give an average value of 2.3 ± 0.4 × 10^{-2} $cm^3 \cdot cell^{-1}$ ($n = 8$). Assuming the cells were spherical, the surface area of a cell with this volume is 8.4 ± 0.7 × 10^{-8} $cm^2 \cdot cell^{-1}$. Chl *a* was estimated according to Wintermans and de Mots (14) and an average value of 0.33 ± 0.05 μg Chl $a \cdot cell^{-1}$ ($n = 8$) was calculated.

Photosynthetic O_2 Evolution. This was measured in an O_2 electrode chamber (Hansatech, England).

Mass Spectrometric Studies. Monitoring of dissolved CO_2 species in aqueous algal suspensions was achieved through the

[1] Abbreviations: C_i, inorganic carbon (CO_2 + HCO_3^- + CO_3^{2-}); CA, carbonic anhydrase.

use of a stirred glass cuvette aqueous inlet system, attached to a mass spectrometer as previously described (2). Algal suspensions were illuminated (400 μmol photons $m^{-2} \cdot s^{-1}$) with a quartz halogen projector lamp.

Calibration of the instrument with CO_2 was achieved by bubbling air of a known CO_2 partial pressure through the liquid in the cuvette and calculating dissolved CO_2 using the Henry constant. The distribution between HCO_3^- and CO_2 was calculated by injecting known amounts of $NaHCO_3$ into the cuvette and measuring the appearance of CO_2. This measured distribution was used to calculate the total C_i uptake or evolution represented by a change in CO_2.

Uniformly labeled [^{18}O]$NaHCO_3$ was prepared by dissolving $NaHCO_3$ in [^{18}O]H_2O (99% enrichment), and allowing 3 d for the label in the H_2O and HCO_3^- to reach isotopic equilibrium. The mixture was then frozen and freeze-dried to recover the solid salt.

Carbonic Anhydrase Measurements. Cells were harvested by centrifugation as described above. The cell pellet was resuspended in extraction buffer (100 mM Bicine [pH 8.0], 1 mM EDTA, 5 mM DTT), and ruptured by passing them twice through a French pressure cell (10,000 p.s.i.). The homogenate was spun at 15,000*g* for 20 min and the supernatant retained for assay. The pellet showed no detectable CA activity (data not shown). Assays were performed in a glass water-jacketed cuvette (0°C) and the reaction monitored with a glass pH electrode. The assay was 3 ml reaction buffer (20 mM Veronal [pH 8.2], 5 mM DTT), 0.3 ml of extract or extraction buffer, and 1.5 ml of CO_2 saturated water (0°C). The time for the pH drop between 7.9 and 6.9 was monitored.

RESULTS

The Model. *Assumptions.* A simple model of photosynthesis can be constructed if one considers *Synechococcus* as a single spherical compartment, into which C_i is actively accumulated, fixed as CO_2 into glycerate 3-P and continually effluxing as CO_2 (Fig. 1). In trying to quantitatively define various parts of this system the following assumptions must be made.

(a) How is C_i entering the cell? The simplest assumption is that it is entering as HCO_3^- (——). It is, however, possible considering evidence from active species experiments with *Synechococcus* (2) to suggest that a number of other possibilities exist (. . .): (i) free CO_2 may be actively taken up directly and enter the cells as CO_2 OR HCO_3^- (ii) HCO_3 may be converted to CO_2 externally and enter as CO_2.

Considering the evidence available, it is reasonable to suggest that in whatever form C_i is taken up, it enters the cell as HCO_3^-.

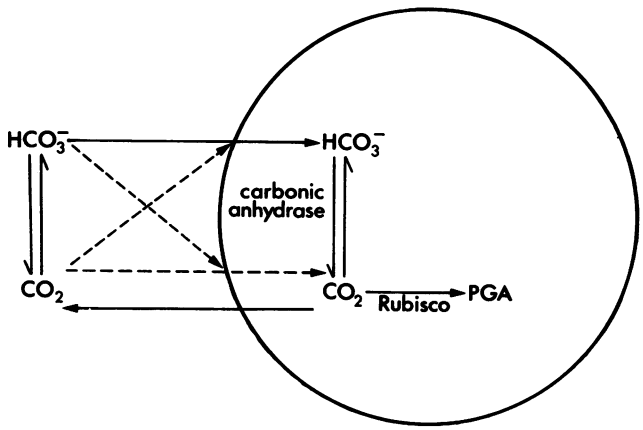

FIG. 1. Schematic model showing possible fluxes of CO_2 and HCO_3^- into and out of a *Synechococcus* cell.

This stems from consideration of: (i) the efflux of CO_2 from *Synechococcus* cells following illumination (2; Fig. 4, top) can only be explained if HCO_3^- is accumulated from the external medium at a rate in excess of net CO_2 fixation; (ii) it is energetically more feasible to construct models for active HCO_3^- uptake, than for CO_2 accumulation, and experiments measuring hyperpolarization of cyanobacterial cells upon addition of external HCO_3^- suggest a primary electrogenic HCO_3^- pump (8).

Thus it will be assumed at present that C_i enters the cell as HCO_3^-, and that this is largely derived from external HCO_3^-.

(b) Once HCO_3^- is inside the cell, then it can only be fixed by RuBP carboxylase upon conversion to CO_2. In this step CA is most likely to play a role. Hence it will be considered low varying amounts of CA may affect the size of the internal HCO_3^- pool necessary to support CO_2 fixation by RuBP carboxylase.

(c) C_i is largely leaving the cell, under steady state conditions, as CO_2, by passive diffusion determined by the concentration gradient across the membrane and the conductance to CO_2. This neglects the possibility that C_i species may be effluxing via reversal of the C_i transport system.

Balance between Bicarbonate Transport, CO_2 Fixation, and Cell Conductance to CO_2. The relationship between HCO_3^- transport into the cell (V_t), CO_2 fixation (V_c), and conductance of the cell to CO_2 efflux (g) may be derived at the point where CO_2 fixation is just saturated with external C_i. At this point it can be assumed that CO_2 inside is saturating for RuBP carboxylase (1000 μM or 5 K_m (CO_2) [1]). Given that CO_2 is small externally compared to this internal level, the CO_2 gradient will be 1000 μM. Under steady state conditions,

$$V_t = V_c + g \times \Delta C \times A \times 10^{-3} \quad (1)$$

and

$$g = \frac{V_t - V_c}{\Delta C \times A} \times 10^3 \quad (2)$$

where V_t is in mol $cell^{-1}$ s^{-1}, V_c is mol $cell^{-1} \cdot s^{-1}$, $\Delta C = 10^{-3}$ mol l^{-1}, A = surface area per cell (cm^2 $cell^{-1}$), and g = cell CO_2 conductance in (cm s^{-1}).

Cell size parameters for *Synechococcus* are given in "Materials and Methods". Using these numbers combined with measured CO_2 fixation rates, this model can be quantified for a range of conditions. Figure 2 presents the relationship between V_c, V_t, and g for two extremes of CO_2 fixation (18–55 $\times$ 10^{-19} mol $cell^{-1}$ s^{-1}). Cell conductance to CO_2 diffusion out (g) is allowed to vary from $10^{-3} - 10^{-6}$ cm s^{-1}, and the ratio of V_t/V_c is calculated over this range (assuming internal CO_2 to be 1000 μM). Also presented in this same figure is the O_2 gradient, which would be expected to exist between the cell and the medium, assuming net O_2 production is equal to V_c, and O_2 conductance is the same as CO_2 conductance.

At cell conductances above 10^{-4} cm s^{-1}, the ratio of V_t to V_c increases markedly. Depending on the energetic cost of transport, it would seem inefficient for the cell to operate in this region. Indeed, to limit V_t to < 2 V_c, g would need to be about 10^{-5} cm s^{-1}. A difficulty that the cell would encounter in achieving a suitable balance of V_t to V_c, is the buildup of O_2 within the cell. The O_2 gradient rapidly increases to levels in excess of one atmosphere partial pressure with g less than 5 $\times$ 10^{-5} cm $\cdot s^{-1}$, depending on the absolute value of V_c.

Carbonic Anhydrase Levels. Internal CA will affect the rates of CO_2 hydration and HCO_3^- dehydration within the cell. Under steady state equilibrium conditions the net rate of CO_2 production within the cell will be given by (k_1 [HCO_3^-] $-$ k_2 [CO_2]$\cdot$ vol), where $k_1 = 10^{(-pH)} \times 10.47 \times 10^4$ s^{-1}, $k_2 = 3.72 \times 10^{-2}$ s^{-1} (11), and vol is the internal cell volume ($l \cdot cell^{-1}$). Given that this will equal the rate of HCO_3^- transport into the cell (V_t), given by equation 1, then the internal [HCO_3^-] which will accumulate

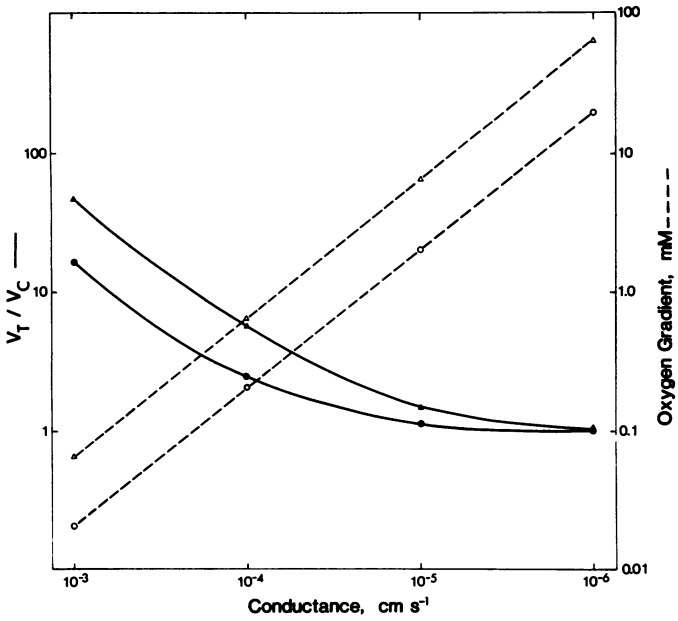

FIG. 2. Possible relationship between the ratio of C_i transport (V_t) to carbon fixation (V_c) given various cell conductances to CO_2. Data are derived from model calculation as described in text, for two V_c values (18 [●] and 55 [▲] × 10^{-19} mol $cell^{-1} \cdot s^{-1}$). Also shown is the oxygen gradient (- - -) which would develop between cell and bulk medium for each of these conditions.

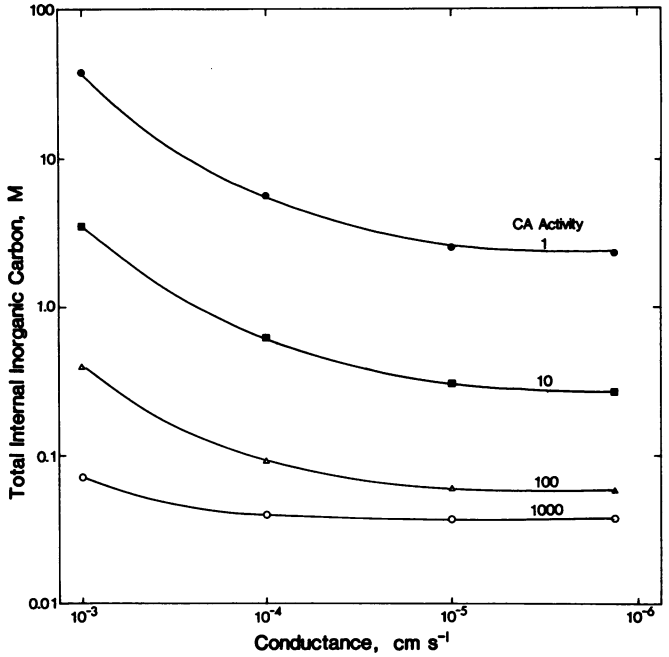

FIG. 3. Effect of various CA levels on the predicted internal HCO_3^- levels developed within the cell, for varying values of cell conductance to CO_2. Carbon fixation is assumed to be 55 × 10^{-19} mol $cell^{-1} \cdot s^{-1}$ and CA levels are expressed as the speed of interconversion of C_i species, relative to the uncatalyzed condition. Calculations are described in the text.

in order to support CO_2 saturated rates of photosynthesis may be calculated. Carbonic anhydrase is simulated by multiplying k_1 and k_2 by some constant representing the relative rate of interconversion of CO_2 and HCO_3^- relative to the uncatalyzed condition. Internal [HCO_3^-] has been calculated allowing cell CO_2 conductance (g) and CA levels to vary (Fig. 3).

Without CA inside the cell, then HCO_3^- levels would have to build up to high levels in order to support CO_2-saturated photosynthesis. The level decreases as conductance (g) decreases, but asymptotically approaches about 2.0 M internal HCO_3^-. Increasing the interconversion rate from 1- to 1000-fold obviously results in decreased internal HCO_3^-. Measured internal C_i pools in *Synechococcus* and other cyanobacteria at CO_2 saturation, appear to be in the region of 30 to 60 mM (2, 7, 9). For cell conductances of 10^{-4} to 10^{-5} cm s^{-1}, these levels are achieved in the model for an increase of interconversion of 100- to 1000-fold. Thus, there is an obvious need in this model for CA to allow the maintenance of reasonable HCO_3^- levels inside the cell. These calculations assume that the internal pH is 8.0. Obviously, the magnitude of the HCO_3^- pool necessary to sustain a particular rate of conversion to CO_2 will depend on the true pH. If the pH was lower than this, then calculated HCO_3^- would be lower.

Measurement of Internal CA. *In Vitro Assay.* A problem with assigning a role for CA in photosynthesis models of cyanobacteria, is that conflicting evidence exists as to the presence or absence of CA in these cells. Ingle and Coleman (6) measured low but detectable levels of CA in low CO_2-grown *Coccochloris peniocystis* which declined when cells were adapted to high CO_2. More recently, studies with *Anabaena variabilis* (7) have failed to detect any activity in low CO_2-grown cells, even though the CA inhibitor, ethoxyzolamide, affected the affinity of photosynthesis for C_i.

Measurement of CA activity in low CO_2-grown cells of *Synechococcus* sp. shows low but detectable presence of a factor speeding up the interconversion of CO_2 and HCO_3^-. In two experiments this activity was measured as 43 and 52 WA units · mg^{-1} Chl a. Taking the average Chl *a* and volume per cell (see "Materials and Methods"), this activity can be confined to the internal cell volume and would result in an increase in the rate of interconversion between CO_2 and HCO_3^- of 350- and 290-fold, respectively. This increased interconversion can be directly compared to the effect of different levels of CA on the operation of the photosynthetic model presented in Figure 3. Activity levels fall in the region sufficient to promote a 100- to 1000-fold increase in internal interconversion rate.

In Vivo Assay. A technique for detecting CA within photosynthesizing cells is by the use of ^{18}O labeled CO_2 and HCO_3^-. In normal seawater medium at pH 8.0 and above, the rate of exchange of ^{18}O out of C_i species into water is relatively slow (13), taking of the order of 1 h or more to reach isotopic equilibrium. This exchange can be measured by monitoring the doubly, singly, and unlabeled species of CO_2 in solution, mass spectrometrically. On a background of slow exchange then, cells can be injected into media and their effects on the overall rate of interconversion of CO_2 and HCO_3^- can be assessed.

If CA is located within the cells, and both CO_2 and HCO_3^- are freely permeable into this space, then adding cells will increase the rate of interconversion detected externally. If, however, only CO_2 is permeable, then little effect of adding cells would be expected. If HCO_3^- access to the internal CA is mediated by a light-dependent pump, then very different results may be expected. In this case, when the light is switched on HCO_3^- will accumulate inside the cells, where it will rapidly exchange with CO_2 and H_2O and lose ^{18}O label. The CO_2 which diffuses back from the cell will be largely unlabeled with respect to ^{18}O. An experiment testing the effect of cells on the exchange of ^{18}O from CO_2 species in solution is shown in Figure 4.

In the absence of cells a steady state rate of exchange of ^{18}O between isotopic species of CO_2 and HCO_3^- is established. With the levels of the various species used here it is evident that the decrease in doubly labeled CO_2 (Fig. 4, 48) is accompanied by an increase in singly (Fig. 4, 46) and unlabled CO_2 (Fig. 4, 44).

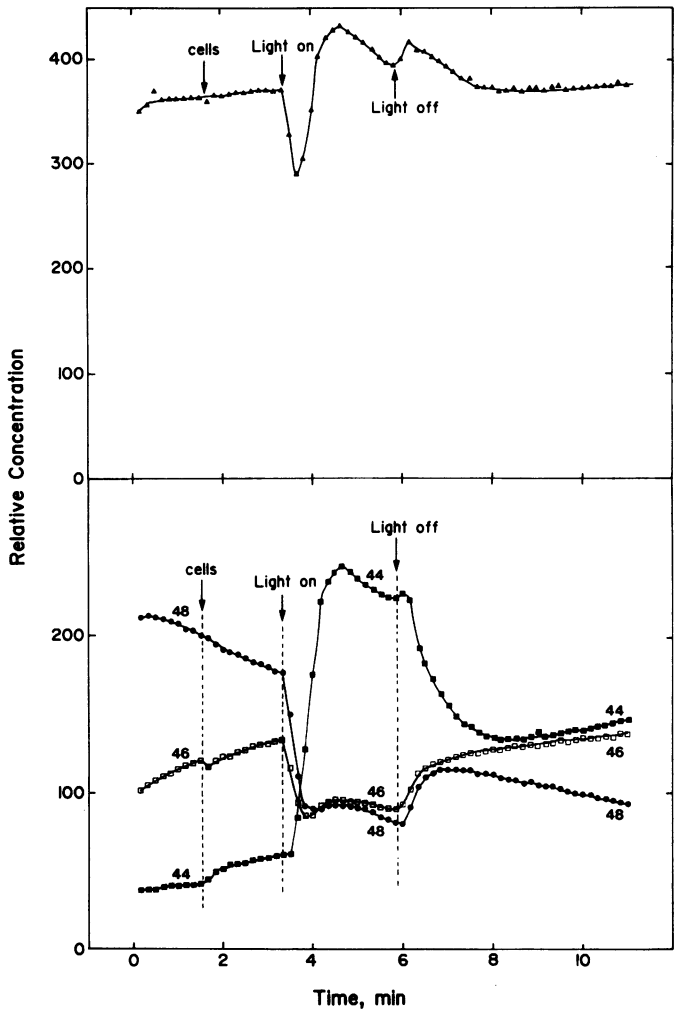

FIG. 4. Change in the relative concentration of [^{18}O] CO_2 species in solution during a time course. The time course involved addition of cells (8 μg Chl·ml^{-1}) in the dark, switching on the light, and then darkening. The change in total CO_2 species is shown in the upper box, while changes in the three individual species are shown below. The total concentration of C_i species was initially 0.5 mM.

Addition of cells (in the dark) to this medium caused little change in the exchange rate, apart from adding a small amount of unlabeled CO_2 with the addition of the cells. A dramatic effect, however, is seen when the light is switched on. There is an uptake of doubly and singly labeled CO_2 species within the first 30 s, followed by a slight increase, which eventually settles down to a steady decrease in both species. Unlabeled CO_2, however, shows no initial uptake in the first 30 s, but instead shows a massive increase over the period that slight increases are seen in the other two species. There is a peak in unlabeled CO_2 level, followed by a decline over the rest of the light period. When the light is switched off there is an increase in the level of labeled species until an exchange pattern similar to that before the light is switched on is reached, with 48 decreasing and 46 increasing. Unlabeled CO_2, however, shows a slight increase followed by a dramatic decrease over the following 3 min until a steady state rate of increase is established, similar to that occurring before the light is switched off.

In all, the events seen here are consistent with cells having a light dependent HCO_3^- pump which accumulates HCO_3^- in a region of rapid exchange between CO_2 and HCO_3^-. The unlabeled CO_2 evolution would be a result of exchange of label within the cell and diffusion of unlabeled CO_2 out of the cell. The slight increase in labeled species following initial uptake in the light could be the result of less than complete exchange of ^{18}O out of CO_2 within the cell, thus leading to some labeled CO_2 evolution, or a decline in CO_2 uptake following the initial transient after turning on the light. Initial uptake of CO_2 species is presumably due to some direct CO_2 uptake process as has been seen previously (2). An increase in labeled species following turning off the light could be due to labeled CO_2 being evolved from the cell and/or a simple reequilibration of species in solution following the cessation of direct CO_2 uptake. The decline in unlabeled CO_2 is consistent with a gradual decline in the rate of evolution of unlabeled CO_2 into the medium as the internal pool of C_i is depleted.

This experiment shows there is strong evidence for implicating the existence of a light-dependent HCO_3^- influx mechanism, together with rapid equilibration of CO_2 and HCO_3^- within the cell.

Effects of Ethoxyzolamide. The CA inhibitor, ethoxyzolamide, has been shown to have a dramatic effect on photosynthesis in air-adapted green algae. It decreases their affinity for external C_i without altering CO_2 saturated photosynthesis, thus making them more like high CO_2 grown cells (3). As the two previous *in vitro* and *in vivo* experiments suggest that CA activity does exist in air-adapted cells of *Synechococcus* sp., an effect of ethoxyzolamide might be expected on these cells. The response of photosynthetic O_2 evolution to C_i in these cells has proven to be unaffected by ethoxyzolamide levels from 10 to 100 μM (data not shown). This result was somewhat unexpected considering the earlier data, and the previously reported inhibition of CO_2-limited photosynthesis in the cyanobacterium *Anabaena variabilis* (7).

Measurement of C_i Uptake, Efflux, and Pool Sizes. To obtain a quantitative understanding of the performance of the cell in relation to the proposed photosynthesis model, measurement of parameters of C_i uptake, efflux, and pool sizes, together with photosynthesis is necessary.

Measurement of these parameters can be achieved by monitoring the uptake and efflux of CO_2 from cells under appropriate conditions. Cells in seawater medium, and in the absence of any added external CA, show a pattern of CO_2 uptake following illumination and darkness, which is similar to the change in total CO_2 species seen in Figure 4. Under these conditions, CO_2 and HCO_3^- are not in rapid equilibrium, so changes in CO_2 are somewhat independent of changes in HCO_3^-. If CA is added then a different time-course is obtained (Fig. 5). Under these conditions, the CO_2 signal is representative of the total C_i species.

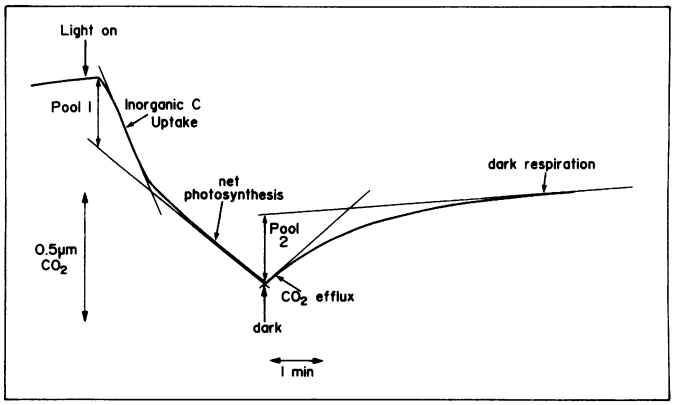

FIG. 5. Time course of changes in CO_2 concentration in an algal suspension (1.5 μg Chl·ml^{-1}) showing effects of light and dark transients. CA at 0.2 mg·ml^{-1} was included. Initial CO_2 was 2.1 μM. The rationale for the drawing of the shown tangents and pool 1 and pool 2 regions are described in the text.

The time-course has three distinct phases. Following illumination, there is a 15- to 20-s delay in CO_2 uptake, followed by a linear phase of CO_2 uptake for the next 30 to 90 s, depending on the C_i level. This uptake phase slows down and is followed by a slower and longer second period of linear CO_2 uptake. Following darkness, CO_2 uptake is replaced by CO_2 evolution, which declines over a 4- to 6-min period to the level of dark respiration. The first CO_2 uptake phase can be attributed to the uptake of C_i into a pool within the cell. As this pool is filled, this net uptake will be replaced by a steady rate of net CO_2 fixation, as seen in the second phase. When the light is turned off, presumably C_i uptake and CO_2 fixation is stopped and the inorganic carbon pool will be released to the medium. The initial rate of C_i release in the dark most probably represents the CO_2 evolution occurring continuously in the light prior to darkness.

Thus, the following measurements may be made from such a time course. (a) The initial slope of the first phase of CO_2 uptake can be taken as an estimate of C_i transport into the cell. (b) The slope of the second region of uptake can be taken as a measure of steady state net CO_2 fixation. (c) The initial slope of the efflux in the dark, minus dark respiratory CO_2 output, can be used as an estimate of C_i leakage from the cell just before the light was turned off. (D) Steady state C_i uptake in the light just prior to darkness can be estimated as net CO_2 fixation plus C_i efflux. (e) Internal C_i pool sizes can be estimated from two regions of the time-course: (i) If the net CO_2 fixation slope is extrapolated back to the light-on time, then the drop in CO_2 from light-on to the extrapolated CO_2 fixation, gives an estimate of the amount of C_i sequestered by the cell (pool 1). (ii) Conversely, if dark respiration is extrapolated back to the light-off time, then the distance pool 2 gives an an estimate of the C_i released into the medium by the cell, following darkness.

If both these pools are partitioned within the estimated cell volumes, then the concentration of C_i within the cell can be calculated.

Experiments were conducted in which air-grown cells of *Synechococcus* were exposed to varying C_i concentrations and the parameters described above in dark-light-dark time courses measured. As a result of the absolute sensitivity of the CO_2 measurement and the high affinity of the cells for CO_2, these experiments could only be conducted at CO_2 concentrations at or above saturating for photosynthesis. Once subsaturating levels were reached, the cells used up most of the inorganic carbon before a complete time course could be achieved.

Estimates of four flux parameters at different external CO_2 concentrations are given in Figure 6. These are net CO_2 fixation, C_i evolution and the two estimates of gross C_i uptake (*i.e.* initial uptake and C_i evolution + net CO_2 fixation). At CO_2 levels which are just saturating for net CO_2 fixation, total C_i uptake by either estimate exceeds net fixation by about 30%. As CO_2 increases above this level inorganic carbon uptake continues to increase, despite the saturation of photosynthetic CO_2 fixation. Both estimates saturate at around 1.5 μM CO_2 at levels which are some 55 to 100% in excess of CO_2 fixation. Estimates of total C_i uptake are higher for the initial uptake parameter than for evolution plus net uptake by about 40%; however, both show the same general response to external CO_2.

The release of C_i in the dark period has been assumed here to represent the leakage rate occurring in the light prior to darkness. It is possible, however, that several changes occurring in the dark may make this invalid. Transient changes leading to depolarization of the plasmalemma and decreased internal pH may lead to an increased rate of C_i release relative to the light. Similarly, if significant leakage is occurring via a reversal of the C_i transport system, then a decrease in energy supply in the dark may alter the efflux via this route. The similarity of estimates of leakage from both the initial C_i uptake in the light and its release in the dark indicates that such considerations may not be of great significance.

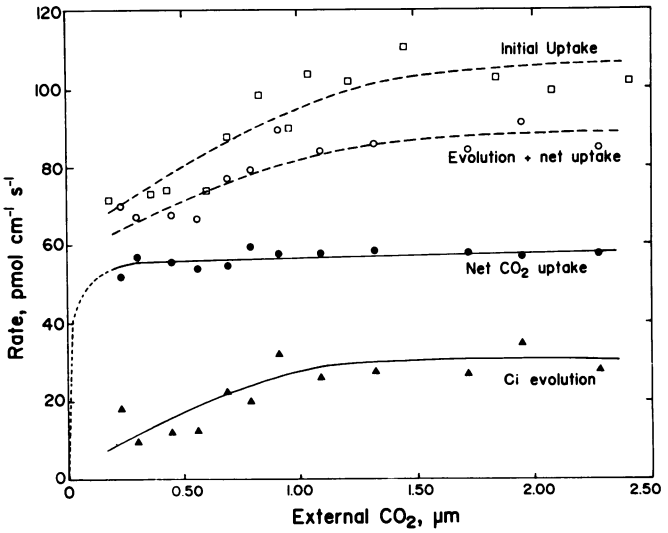

FIG. 6. Values for net CO_2 fixation, total C_i uptake and C_i evolution at varying external CO_2 in *Synechococcus* sp., obtained from light/dark time courses similar to those shown in Figure 5. Calculation of the data is described in the text. All data were collected for a single set of cells at a concentration of 0.8 μg Chl·ml^{-1}. Different initial CO_2 concentrations were achieved by both varying the amount of added $NaHCO_3$ and also by performing several time courses on the same cell suspension, allowing the cells to deplete the medium of CO_2. The cell parameters given in "Materials and Methods" were used to express the flux rates on a cm^{-2} cell surface basis.

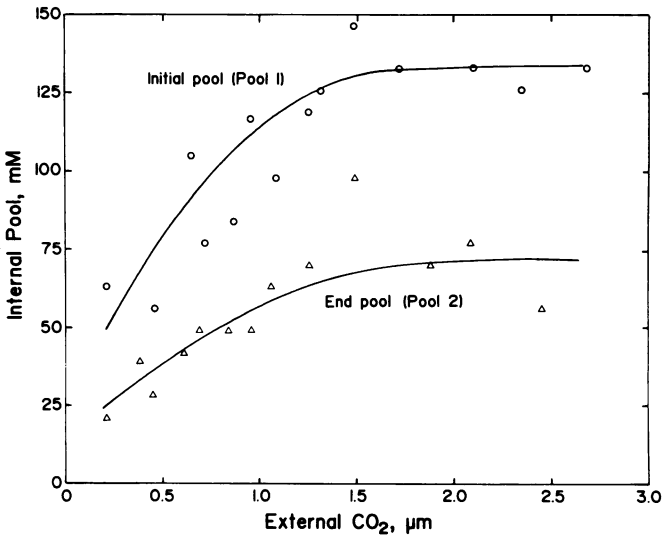

FIG. 7. The response of internal C_i pool to external CO_2 in *Synechococcus* sp. Two pools (pool 1 and pool 2) are estimated from the data in Figure 6 as described in the text and in Figure 5.

The internal C_i pools (pool 1 and pool 2) calculated from these experiments are given in Figure 7. The initial pool 1 estimates prove to be some 100% higher than the end pool 2 values. At just saturating external CO_2, internal (C_i) is estimated at around 50 mM from pool 1 and 25 mM for pool 2. Both pools respond to increasing external CO_2 in a similar fashion and saturate at around 1.5 μM CO_2. This agrees well with the response of total C_i uptake which, ultimately, these pools are derived from. Significantly, the variation in the size of the pools are correlated with the total C_i uptake values measured at the beginning and

end of the time course. Rather than being an artefact of measurement, C_i uptake may indeed be initially faster in the absence of CO_2 fixation than at the end of the light period, and consequently the steady state internal pool sizes are larger at the beginning than the end of this period.

From the end pool and initial C_i efflux measurements, it is possible to make estimates of the cell conductance to CO_2. This assumes (a) the cell volume and surface dimensions given in "Materials and Methods"; (b) that CO_2 is the major species effluxing; (c) that CO_2 and HCO_3^- are in rapid equilibrium internally, and hence that the actual internal concentration of CO_2 can be derived from the pool sizes and an estimated internal pH, using the pK_1 of H_2CO_3 (6.29). These conductance estimates, calculated for three assumed internal pH values (pH 7.5, 8.0, and 8.5), range from $6.07 \pm 0.34 \times 10^{-6}$ at pH 7.5 to $5.72 \pm 0.32 \times 10^{-4}$ at pH 8.5.

DISCUSSION

The technique of monitoring CO_2 (in the presence of CA) in solution during light/dark time courses, has helped quantify the steady state fluxes of C_i in photosynthesizing cells of *Synechococcus*. At levels of external C_i which are just saturating for photosynthesis, total C_i uptake is ony about 30% higher than net CO_2 fixation. This means that of the C_i being taken up and concentrated by the cell, only about 30% is leaking out into the external medium. This leakage becomes higher as C_i increases above levels which are saturating for fixation. This is due to a continuing response of the uptake system to increasing C_i, while fixation remains constant. The level of 30% leakage indicates that the system is relatively tight in terms of coupling uptake to CO_2 fixation. Similar leakiness has been estimated for the higher plant C_4 CO_2 concentrating system (4).

Internal pool size estimates from these experiments are in the range of values estimated in cyanobacteria, using silicone oil-centrifugation techniques. In these studies, pool sizes of 15 to 50 mM for saturating external C_i have been obtained (2, 7, 9). The observation that the initial pool estimate is considerably higher than the pool estimated after a period of steady state photosynthesis may be related to changes in the rate of C_i uptake and CO_2 fixation during the time course. Initially, in the absence of significant CO_2 fixation and competition for energy, transport will probably be high. This, coupled to reduced CO_2 fixation, would lead to high internal pools. This balance would adjust as competition for energy changed and CO_2 fixation increased. The pool size at the end of the time course is probably more relevant in considering the pool sizes which are necessary to support photosynthesis. Likewise, the uptake measurements estimated at this time are also most pertinent to assessing the balance between transport and fixation during steady state photosynthesis. It is significant that transport estimates are higher in the initial period than after steady state photosynthesis. This would support the interpretation of why pool sizes vary as discussed above.

Carbonic anhydrase activity is readily detected in intact cells (Fig. 4) using the ^{18}O labeled CO_2 technique. This, coupled to small but detectable levels of assayable activity in broken cells, suggests that it may be reasonable to assume that HCO_3^- and CO_2 may be near chemical equilibrium within the cell. This finding is in contrast to previous interpretations that C_i species may be far from chemical equilibrium due to an absence of CA (7).

Calculation of cell conductance values for passive CO_2 efflux shows that the cell may have interesting properties with regard to gas diffusion. At reasonable estimates of internal pH (pH 7.5–8), it would appear that conductance values of around 10^{-5} cm s^{-1} are necessary to explain the leakage rates of CO_2 that are measured (Fig. 6). This value is extremely low compared to estimates of other biological membrane systems; however, it is similar to an estimate of CO_2 conductance made in the unicellular green algae, *Dunaliella salina* (15). These conductances are calculated assuming that only CO_2 is effluxing, rather than HCO_3^-. If HCO_3^- may also leave the cell via a reversal of the C_i transport system, then these values will be overestimates.

The simple model developed for C_i transport and photosynthesis, agrees remarkably with the actual measurements made on photosynthesizing cells. For a ratio of transport to fixation (V_t/V_c) of less than 2, the model predicts that cell conductance to CO_2 leakage would have to be less than 10^{-4} cm·s^{-1} (Fig. 2). For 30% leakage, this would be closer to 10^{-5} cm s^{-1}. This is very close to the estimates of conductance from pool size and CO_2 efflux measurements, assuming an internal pH of 8.0. The presence of CA is in agreement with the apparent necessity in the model, if total internal C_i is not to reach unmanageably high levels (Fig. 3). The measured values of internal activity sufficient to promote a 300-fold increase in interconversion rate, satisfy the requirements of the model, as this would lead to better than 90% equilibration between CO_2 and HCO_3^- species. The lack of an effect of ethoxyzolamide on C_i usage is inconsistent with measured CA activity, the model and previously reported effects on the cyanobacterium *A. variabilis* (7). The reason for this is not clear at present.

While the model based on HCO_3^- transport into the cell and CO_2 efflux outward does fit the available experimental data, two features of both its predictions and the measurements from cells need some consideration. The prediction and measurement of apparently very low values for conductance to CO_2 is very hard to explain in terms of the known properties of biological membranes and diffusion layers. This conductance is roughly equivalent to an unstirred water layer thickness of 1 cm and compares to total CO_2 conductance values in leaves of 10^{-1} to 10^{-2} cm s^{-1}. Assuming that CO_2 and O_2 diffuse similarly in these cells, then these low conductances will pose severe problems to the escape of photosynthetically evolved O_2. Calculations show that at 10^{-5} cm s^{-1}, O_2 would build up internally to levels between 2 and 7 mM (Fig. 2). This is equivalent to partial pressures of 1.7 to 5.9 atmospheres of oxygen. This would certainly pose toxicity problems to the cell. Such a conductance would also pose problems to oxygen availability for respiration in the dark.

The resolution of the apparently low conductance values both in the model and experimental data, remain to be resolved; however, it is clear that the CO_2 concentrating system in these cells operates relatively efficiently with leakage of CO_2 not in excess of the C_4 mechanism in higher plants. This would minimize the energy expenditure involved in concentrating CO_2 at the site of carboxylation. Measured parameters of C_i fluxes in these cells can be simulated by the proposed simple HCO_3^- transport model. This, however, is not proof and resolution of the actual mechanism of CO_2 concentration remains forthcoming.

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