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Communication

Simultaneous Transport of CO_2 and HCO_3^- by the Cyanobacterium *Synechococcus* UTEX 625[1]

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ABSTRACT

A mass spectrometer was used to simultaneously follow the time course of photosynthetic O_2 evolution and CO_2 depletion of the medium by cells of the cyanobacterium *Synechococcus leopoliensis* UTEX 625. Analysis of the data indicated that both CO_2 and HCO_3^- were simultaneously and continuously transported by the cells as a source of substrate for photosynthesis. Initiation of HCO_3^- transport by Na^+ addition had no effect on ongoing CO_2 transport. This result is interpreted to indicate that the CO_2 and HCO_3^- transport systems are separate and distinctly different transport systems. Measurement of CO_2-dependent photosynthesis indicated that CO_2 uptake involved active transport and that diffusion played only a minor role in CO_2 acquisition in cyanobacteria.

Cyanobacteria such as *Anabaena variabilis* (1, 10, 20), *Coccochloris peniocystis*, (7, 12, 13), *Synechococcus* sp. (4), and *Synechococcus leopoliensis* UTEX 625 (7, 8, 14, 16) possess mechanisms for the active transport of both CO_2 and HCO_3^-. The ability to experimentally distinguish between the two transport processes, however, is complicated by the fact that CO_2 and HCO_3^- are readily interconvertible. To circumvent this problem many of the assay procedures for CO_2 and HCO_3^- transport (1, 4, 7, 12, 13, 19) involve transient measurement of activity and are conducted under experimental conditions which favor transport of the DIC[2] species of interest. These protocols, however, generally preclude the simultaneous measurement of the rate of transport of the other species of DIC. Consequently, there is uncertainty as to the relative contribution of each species of DIC to total DIC uptake and the extent to which each transport system functions *in situ*. In the present communication, we demonstrate that CO_2 and HCO_3^- are simultaneously and continuously taken up during steady state photosynthesis by the cyanobacterium *Synechococcus* UTEX 625. Implications regarding the mechanism of transport are briefly explored.

MATERIALS AND METHODS

Synechococcus UTEX 625 (*Synechococcus leopoliensis* University of Texas Culture Collection), also known as *Anacystis nidulans* (17, 18), was grown on air (0.05% v/v CO_2) in unbuffered Allen's medium (2) as described previously (8). Cells were harvested for experiments when the [Chl] reached 6 to 9 μg/ml. At harvest, the pH of the culture medium was between 9.5 and 10.2. Prior to experiments, the cells were washed three times by centrifugation (1.0 min at 10,000*g*, Beckman Microfuge B) in 25.0 mM BTP/23.5 mM HCl buffer (pH 8.0), which contained only contaminant levels of DIC (15 μM) and Na^+ (5 μM) (17). Subsequently, 5 ml of cell suspension was placed in a thermostated (30°C) glass reaction vessel and allowed to equilibrate for 3 to 5 min in the dark. The vessel was closed to the atmosphere during experiments. Dissolved CO_2 and O_2 in the cell suspension were alternately measured at 3 s intervals using a magnetic sector mass spectrometer (VG Gas Analysis, Middlewich, England, model MM14-80 SC) equipped with a membrane inlet system (9). Data were stored on magnetic discs for later retrieval and analysis. Alternatively, when a single dissolved gas was measured, the output from the mass spectrometer was directed to a chart recorder. The mass spectrometer was calibrated for CO_2 by adding a known volume of 100 mM K_2CO_3 to a darkened cell suspension. The CO_2 concentration generated (pH 8.0, 30°C) was calculated from the equations of Buch (5) and corrected for ionic strength (I = 0.025 M) (15). The added K_2CO_3 also served as the DIC source for photosynthesis. Calibration for dissolved O_2 was achieved using the catalase-H_2O_2 procedure (6).

Calculations. Theoretical rates of photosynthesis based on the passive uptake of CO_2 alone were calculated using the equations described by Miller (11). For passive uptake it was assumed that the CO_2 concentration required to attain one-half the maximum rate of photosynthesis was equal to the K_m (CO_2) of isolated cyanobacterial Rubisco (250 μM) (3). A measured value of 300 μmol $O_2 \cdot mg^{-1}$ Chl $\cdot h^{-1}$ was taken as the maximum rate of photosynthesis (V_{max}). The apparent first order rate constant for photosynthesis, kp', was calculated from the equation

$$kp' = V_{max}/K_m(CO_2) \quad (1)$$

For the analysis we varied $K_m(CO_2)$ between 250 μM CO_2 and 1 nM CO_2.

The maximum rate of CO_2 supply, occurring from HCO_3^- dehydration, to cells in a closed system was calculated in two ways: method A from the equations of Miller and Colman (13) or method B from the equation

$$\frac{d[CO_2]}{dt} = k_d\,[HCO_3^-] \quad (2)$$

Use of Eq. 2 was justified since the actual measured level of CO_2 approached zero during experiments; hence the back reaction from CO_2 to HCO_3^- was not a major factor in determining the CO_2 concentration. The value for k_d (0.87×10^{-3} s^{-1}) was determined empirically by using the mass spectrometer to follow

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[2] Abbreviations: DIC, dissolved inorganic carbon (CO_2 + HCO_3^- + CO_3^{2-}); BTP, 1,3-bis [tris (hydroxymethyl) methylamino] propane; CA, carbonic anhydrase; Rubisco: D-ribulose 1,5-bisphosphate carboxylase/oxygenase (EC 4.1.1.39).

the time course of CO_2 appearance in BTP/HCl reaction buffer (pH 8.0, 30°C) of the appropriate ionic strength, following the addition of 1 mM K_2CO_3. Eq. 2 yielded approximately a two-fold lower rate of CO_2 supply than the equations of Miller and Colman (Fig. 1). The difference lies in the fact that ionic strength was taken into account in Eq. 2 but not in the other equations (13).

RESULTS AND DISCUSSION

Simultaneous Transport of CO_2 and HCO_3^-. Figure 1 illustrates a typical time course of CO_2 removal from the medium (pH 8) by cells of *Synechococcus* following illumination in the presence of 55 μM DIC. The first phase of the time course lasted for about 40 s and involved rapid CO_2 depletion of the medium, to a level near zero. In the second phase of the time course, this low level of CO_2 was maintained constant until the cells were darkened. If CA, a catalyst for CO_2-HCO_3^- interconversion, was added to the illuminated cell suspension shortly after attaining phase 2, the extracellular [CO_2] rapidly returned to a level near that found prior to illumination (Fig. 2). These results show that, in the absence of CA, the rapid removal of CO_2 from the medium by the cells caused the CO_2-HCO_3^- system to be drawn far from chemical equilibrium. Second, these results also show that CO_2, rather than CO_2 + HCO_3^-, was selectively removed from the medium during the initial phase of the time course, since a return to a value close to the pre-illumination level of CO_2 (Fig. 2) would not be expected if all the DIC (CO_2 + HCO_3^-) was sequestered within the intracellular pool. Photosynthetic fixation may have accounted for some of the initial consumption of CO_2 (Fig. 1). However, the brevity of the initial phase of uptake and the low rate of photosynthesis, in the absence of Na^+ (Fig. 1), indicated that CO_2 fixation would only be a minor factor in the disappearance of CO_2 from the medium. In fact, we have obtained nearly identical time courses when the cells have been poisoned with iodoacetamide (15), an inhibitor of the Calvin cycle. Thus, the initial removal of CO_2 from the medium by *Synechococcus* represents uptake of CO_2 into the intracellular pool of inorganic carbon.

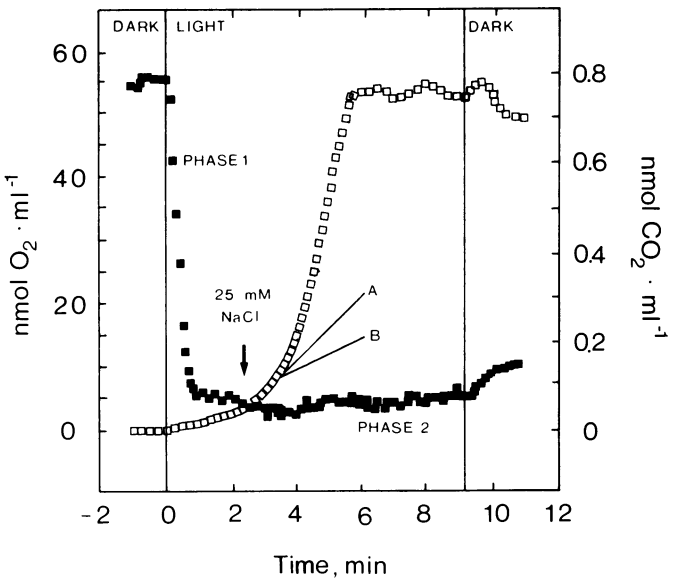

FIG. 1. Simultaneous measurement of photosynthetic O_2 evolution (□) and CO_2 depletion (■) of the medium by *Synechococcus* following illumination in the presence of 55 μM DIC. Cells (12.9 μg Chl·ml^{-1}) were suspended in BTP/HCl buffer (pH 8.0), at 30°C, and light was provided at 600 μE·m^{-2}·s^{-1} PAR. Active transport of HCO_3^- was initiated by the addition of 25 mM NaCl. The rates of photosynthesis (μmol O_2·mg^{-1}·Chl·h^{-1}) prior to and after Na^+ addition were 16 and 128, respectively. Parallel experiments (not shown) demonstrated that the increase in photosynthesis was, in fact, dependent upon Na^+. Solid lines (A, B) represent photosynthesis (O_2 evolution) which could be supported by CO_2 uptake alone, as calculated by method A or B.

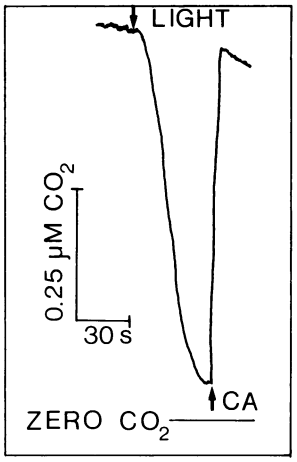

FIG. 2. Time course of CO_2 depletion of the medium by *Synechococcus* following illumination in the presence of 65 μM DIC. Shortly after attaining steady state CO_2 uptake (phase 2), 25 μg·ml^{-1} CA (62.5 Wilbur-Anderson units/ml) was added to the cell suspension. Cells (7.7 μg Chl·ml^{-1}) were suspended in BTP/HCl buffer (pH 8.0), containing 100 μM NaCl at 30°C, and light was provided at 600 μE·m^{-2}·s^{-1} PAR.

The near-zero, nonequilibrium, level of CO_2 found during the second phase of the time course (Fig. 1) was maintained in spite of the fact that extracellular HCO_3^- dehydration served as a means for the regeneration of CO_2 within the system. In light of this consideration, maintenance of a low nonequilibrium CO_2 concentration must be interpreted as indicating continuous uptake of CO_2 by the cells at a rate equal to the HCO_3^- dehydration rate. If CO_2 uptake ceased after attaining phase 2, it would be expected that the CO_2 concentration would return to its equilibrium level. This was clearly not the case (Fig. 1).

Figure 1 also shows the simultaneously recorded time course for O_2 evolution following illumination of the cells. In the absence of Na^+, a rate of 16 μmol O_2·mg^{-1} Chl·h^{-1} was observed and this closely corresponded to the theoretical rate (13 μmol O_2·mg^{-1} Chl·h^{-1}) of CO_2 supply to the system from HCO_3^- dehydration (method B). Addition of 25 mM NaCl to the cell suspension enhanced photosynthetic O_2 evolution eightfold, but had no apparent effect on the rate (phase 2) of CO_2 uptake. Control experiments demonstrated that the increase in photosynthesis was, in fact, a consequence of Na^+ addition (not shown; see 8, 14, 17).

Previous studies (8, 14, 17) have shown that millimolar levels of Na^+ stimulate the active transport of HCO_3^- in *Synechococcus*. The criterion that we used to establish HCO_3^- uptake was to compare the rate of photosynthesis to the maximum rate which could be supported by CO_2 uptake alone (Fig. 1). In this experiment photosynthesis exceeded the CO_2 supply rate calculated from method A, by 5.2-fold or by 9.8-fold as calculated from method B. Thus, the additional DIC required to support the increased rate of photosynthesis must be obtained from the medium through HCO_3^- uptake. The necessity for HCO_3^- uptake in support of photosynthesis is further emphasized by the fact that the CO_2 concentration was near zero just prior to the addition of Na^+ (Fig. 1) and thus cannot be the source of additional C for fixation. Collectively, the data in Figure 1 indicate that both CO_2 and HCO_3^- were simultaneously and continuously taken up

by the cells during steady state photosynthesis at pH 8.

Interaction between CO_2 and HCO_3^- Uptake. It is of interest to note that the initiation of HCO_3^- transport by Na^+ addition had little effect on the concentration of CO_2 maintained in the medium during phase 2. It might be expected that any competitive effect of HCO_3^- transport on CO_2 transport (20) would be manifested as an increase in the steady state (phase 2) level of CO_2 in solution. The fact that the initiation of HCO_3^- transport did not alter the extracellular CO_2 concentration suggests that CO_2 and HCO_3^- were taken up by distinctly different transport systems. This finding is in contrast to data obtained for *Anabaena variabilis* (20), where it was proposed that CO_2 and HCO_3^- interacted at a common site and that the carrier systems for both species of DIC were linked (20). It is possible CO_2 transport was inhibited by HCO_3^- uptake but that the cells possessed sufficient CO_2 transport capacity to maintain the extracellular CO_2 concentration near zero in spite of the inhibition. This explanation, however, was not sufficient since even under conditions where the rate of CO_2 transport was near maximum (high [DIC]) Na^+ addition (HCO_3^- transport) had little effect on the steady state CO_2 concentration in the medium (not shown).

Experiments similar to that shown in Figure 1 have been conducted by Badger and Andrews (4) using a marine species of *Synechococcus*. In both cyanobacterial species, an initial rapid depletion of CO_2 from the medium occurred upon illumination. With the marine species, however, this was followed by a massive efflux of CO_2 from the cells rather than by the establishment of a low and constant extracellular CO_2 concentration (Fig. 1). Consequently, for the marine species, it is difficult to ascertain whether or not inwardly directed active CO_2 transport continued during the CO_2 efflux stage, although it was clear that HCO_3^- transport was occurring (4).

Active Transport of CO_2. The uptake of CO_2 alone, before the addition of 25 mM NaCl, was sufficient to support a low rate of photosynthesis (Fig. 1). However, the observed rate of photosynthesis was some 22-fold higher than expected based on the *in vitro* kinetic characteristics of cyanobacterial Rubisco and the ambient CO_2 concentration passively distributed between cells and the medium (Fig. 3). Although the rate of conversion of HCO_3^- to CO_2 was just adequate to support the observed rate of photosynthesis (Fig. 1), this photosynthesis could only occur if the CO_2 taken up was accumulated within the cells to a level which far exceeded the extracellular concentration. This would require that CO_2 be actively transported across the plasmalemma. In other work (15), we have used the mass spectrometer to measure uptake of CO_2 by *Synechococcus* against concentration gradients in excess of 17,000-fold. The present analysis provides independent evidence for the active transport of CO_2 and indicates that maintenance of even low rates of photosynthesis requires active accumulation of CO_2. Consequently, the diffusive uptake of CO_2 plays a very minor role in the supply of DIC for photosynthesis in cyanobacteria.

Relative Contribution of CO_2 and HCO_3^- Uptake to Photosynthesis. Maintenance of a near-zero concentration of CO_2 (Fig. 1) showed that the rate of CO_2 resupply to the system, from HCO_3^- dehydration, occurred at or near its maximum theoretical rate. This observation in conjunction with the fact that the rate of photosynthesis (O_2-evolution) closely corresponded to the theoretical rate of CO_2 supply, in the absence of Na^+, indicated that little, if any, HCO_3^- was taken up by the air-grown cells through an Na^+-independent (8) mechanism. *Synechococcus* was also capable of reducing the CO_2 concentration to near zero at extracellular DIC concentrations up to 200 μM, both in the absence and presence of Na^+ (HCO_3^- transport) (not shown). Although the actual extent of CO_2 depletion depended upon cell density, these results showed that under certain circumstances the cellular capacity for active CO_2 transport was sufficient to support photosynthesis at rates close to the maximum rate of CO_2 supply (method B) at DIC concentrations up to saturation. Thus, the net contribution made by CO_2 uptake to the supply of DIC for photosynthesis could be defined.

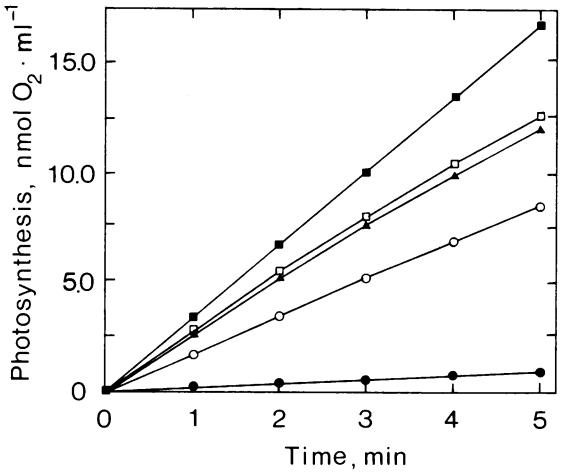

FIG. 3. Comparison between the observed, CO_2-dependent, rate of photosynthesis (■) and that expected (●, ○, ▲, □) if Rubisco uses CO_2 from the medium without transport and subsequent intracellular accumulation. For the analysis, the K_m (CO_2) of Rubisco was taken as 250 μM (●), its measured value (3), and it was assumed to be equal to the $K_{1/2}$ (CO_2) of photosynthesis. Alternatively, a value of 10 μM (○), 1 μM (▲), or 1 nM (□) was used. The V_{max} was held constant at 300 μmol $O_2 \cdot mg^{-1}$ Chl $\cdot h^{-1}$. The CO_2-dependent rate of photosynthesis, prior to Na^+ addition, was 3.4 nmol $O_2 \cdot ml^{-1} \cdot min^{-1}$ (Fig. 1). Other conditions as in Figure 1.

Rates of photosynthesis in excess of the CO_2 supply rate require a direct contribution from HCO_3^- uptake to provide the additional substrate for photosynthesis (Fig. 1). In this case (Fig. 1), after the addition of Na^+, 12.5% of the DIC for photosynthetic fixation would have been supplied through CO_2 uptake while the remainder was supplied through HCO_3^- uptake. The relative contribution of CO_2 or HCO_3^- to photosynthesis would, of course, depend upon experimental conditions. At low levels of extracellular DIC (<100 μM) and/or at alkaline pH (>8.5), Na^+-dependent HCO_3^- uptake was the major means of DIC acquisition for photosynthesis in air-grown cells (8, 16). As the extracellular DIC concentration was increased, CO_2 uptake provided an increasing proportion of the total DIC for photosynthesis as governed by the rate of CO_2 supply and the kinetic characteristics of the transport system. At high DIC concentration (>500 μM, pH 8), where the rate of CO_2 supply is not limiting, it is theoretically possible that all of the CO_2 required for fixation would be acquired through CO_2 transport. Whether HCO_3^- transport continues under these conditions is not known. A putative cessation of HCO_3^- transport at high DIC concentration would require some means of regulating the transport system.

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