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Inorganic Carbon-Stimulated O_2 Photoreduction Is Suppressed by NO_2^- Assimilation in Air-Grown Cells of *Synechococcus* UTEX 625[1]

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The effect of NO_2^- assimilation on O_2 exchange and CO_2 fixation of the cyanobacterium, *Synechococcus* UTEX 625, was studied mass spectrometrically. Upon addition of 1 mM inorganic carbon to the medium, inorganic carbon pools developed and accelerated O_2 photoreduction 5-fold when CO_2 fixation was inhibited. During steady-state photosynthesis at saturating light, O_2 uptake represented 32% of O_2 evolution and balanced that portion of O_2 evolution that could not be accounted for by CO_2 fixation. Under these conditions, NO_2^- assimilation reduced O_2 uptake by 59% but had no influence on CO_2 fixation. NO_2^- assimilation decreased both CO_2 fixation and O_2 photoreduction at low light and and increased net O_2 evolution at all light intensities. The increase in net O_2 evolution observed during simultaneous assimilation of carbon and nitrogen over carbon alone was due to a suppression of O_2 photoreduction by NO_2^- assimilation. When CO_2 fixation was precluded, NO_2^- assimilation inhibited O_2 photoreduction and stimulated O_2 evolution. When the electron supply was limiting (low light), competition among O_2, CO_2, and NO_2^- for electrons could be observed, but when the electron supply was not limiting (saturating light), O_2 photoreduction and/or NO_2^- reduction caused electron transport that was additive to that for maximum CO_2 fixation.

Photoreduction of O_2 in cyanobacteria such as *Synechococcus* UTEX 625 is greatly stimulated when an internal C_i pool is allowed to develop in the light, whether CO_2 fixation is permitted or not (Miller et al., 1988). When CO_2 fixation was inhibited by IAC or glycolaldehyde, net O_2 evolution was not observed, but the C_i-stimulated rates of O_2 evolution and O_2 reduction were nearly equal to the rates of O_2 evolution observed with C_i-saturated photosynthesis (Miller et al., 1988). The occurrence of photochemical quenching with O_2 photoreduction when CO_2 fixation was inhibited clearly indicated that linear electron transport was stimulated by the development of the intracellular C_i pool (Miller et al., 1991; Badger and Schreiber, 1993). The site of O_2 photoreduction is unclear, but based on inhibition with 2,5-dibromo-3-methyl-1,6-isopropyl *p*-benzoquinone and restoration by *N,N,N′,N′*-tetramethyl-*p*-phenylene diamine it was suggested that O_2 was reduced via Fd (Canvin et al., 1990), which would be consistent with results with higher plants (Badger, 1985; Asada and Takahashi, 1987). In this perception of O_2 photoreduction the intracellular C_i pool would in some way stimulate electron flow to Fd in the photosynthetic electron transport chain.

This view, however, was questioned when it was observed that the reduction of artificial electron acceptors for PSI or PSII did not appear to require the development of a C_i pool (Badger and Schreiber, 1993), and it was suggested that the "HCO_3^- may have a direct effect on reactions involved in O_2 photoreduction."

NO_3^- and NO_2^- reduction in cyanobacteria is dependent on light-generated reducing equivalents (Serrano et al., 1981, 1982; Flores et al., 1983; Romero and Lara, 1987), and there seems to be no doubt that NO_2^- is reduced by Fd (Miguel and Lara, 1987). Recently (Mir et al., 1995) we showed that NO_2^- photoreduction is greatly enhanced by the development of the internal C_i pool. That the internal C_i pool stimulated the photoreduction of both O_2 and NO_2^- seemed to indicate again that the C_i pool was influencing electron flow in the photosynthetic electron transport chain and that the reduction of O_2 and NO_2^- (and CO_2) derived electrons from a common (or proximate) intermediate(s) of PSI.

In this study we examined the interaction of NO_2^- assimilation with the C_i-stimulated photoreduction of O_2 in *Synechococcus* UTEX 625 under both noninhibited and inhibited conditions of CO_2 fixation. We provide evidence that at saturating light NO_2^- reduction diminishes primarily O_2 photoreduction, whereas at lower light intensity both O_2 photoreduction and CO_2 fixation are suppressed. The results show that the reduction of O_2, NO_2^-, and CO_2 compete for electrons from PSI and suggest that the intracellular C_i pool acts by stimulating electron flow in the photosynthetic electron transport chain.

MATERIALS AND METHODS

The unicellular cyanobacterium *Synechococcus* UTEX 625 (University of Texas Culture Collection, Austin) was grown with air bubbling (0.036% [v/v] CO_2) in unbuffered Allen's medium at 30°C as described by Espie and Canvin (1987). The growth medium contained 17.6 mM $NaNO_3$.

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Abbreviations: BTP, 1,3-bis[tris(hydroxymethyl)-methylamino]propane; CA, carbonic anhydrase; C_i, dissolved inorganic carbon (CO_2 + HCO_3^- + CO_3^{2-}); E_0, gross O_2 evolution; IAC, iodoacetamide; U_0, gross O_2 uptake.

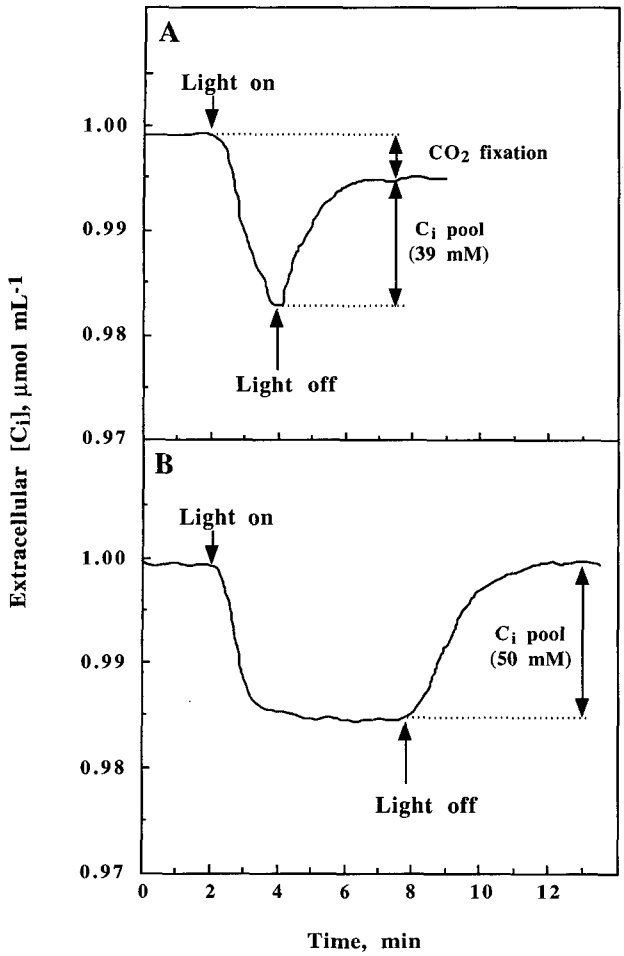

Figure 1. C_i pool formation within the cells of *Synechococcus* UTEX 625 under noninhibited (A) and inhibited (B) conditions of CO_2 fixation. The cells were preincubated in the light to remove C_i from the medium. The lights were turned off and cells were dark adapted for 2.5 min. C_i in the form of $K_2{}^{13}CO_3$ was added to a final concentration of 1 mM in the dark. The lights were turned on and C_i pool formation within the cells was monitored mass spectrometrically as described by Miller et al. (1988). Actinic light was 300 μmol m^{-2} s^{-1} (PAR), Chl *a* was 3 to 5 μg mL^{-1}, and 25 mM NaCl and 25 μg mL^{-1} CA were present in the reaction mixture.

Experimental Conditions

Cells were washed three times by centrifugation (1 min at 10,000*g*, Beckman Microfuge B) and resuspended (5–7 μg Chl mL^{-1}) in 25 mM BTP/HCl buffer, pH 8.0. This buffer contains only 10 to 20 μM C_i when it is kept under N_2 in a serum stoppered flask (Miller et al., 1984). The resuspended cells were placed in a glass chamber at 30°C and 60 μmol m^{-2} s^{-1} light and bubbled with CO_2-free air or nitrogen to remove any remaining C_i. Six milliliters of cell suspension were transferred to the reaction chamber and allowed to reach the CO_2 compensation point after addition of 25 mM NaCl, which is required for HCO_3^- transport (Miller and Canvin, 1985). Illumination was provided by a tungsten halogen projection lamp. NO_2^- was added as KNO_2.

MS

Concentrations of dissolved $^{16}O_2$, $^{18}O_2$, and $^{13}CO_2$ (m/z 32, 36, and 45, respectively) in the 6 mL of cell suspension in the reaction chamber were measured using a magnetic sector mass spectrometer (VG Gas Analysis, Middlewich, UK; model MM 14–80 SC) equipped with a membrane inlet connected to the chamber as described previously (Miller et al., 1988). Calibration for CO_2 was performed by injecting small amounts of $K_2{}^{13}CO_3$ in the stoppered cuvette in BTP/HCl buffer and calculating the equilibrium concentration of CO_2 at 30°C and pH 8 (Miller et al., 1988). The mass spectrometer was also calibrated by injecting the K_2CO_3 into 0.2 N HCl in the reaction cuvette. Calibration for $^{16}O_2$ was made by bubbling 6 mL of the same BTP/HCl buffer used for the incubation of cells with air until equilibrium had been reached and then with nitrogen to obtain a zero O_2 concentration. The value of the concentration of $^{16}O_2$ in equilibrium with air was taken as 240 μM. Calibration for $^{18}O_2$ was made by saturating 6 mL of $^{16}O_2$-free BTP/HCl buffer with 1 mL of $^{18}O_2$ gas by vigorous shaking at 30°C for 15 to 20 min. The O_2 content in the $^{18}O_2$ saturated buffer was then determined mass spectrometrically (Miller et al., 1988).

After the cell suspension had reached the CO_2 compensation point, it was flushed briefly with nitrogen to bring the $^{16}O_2$ concentration to zero. At this point, $^{18}O_2$ was introduced into the cyanobacterial suspension from a bubble that was removed when the $^{18}O_2$ concentration reached about 190 μM. During this time (2–3 min), some $^{16}O_2$ (20–25 μM) and $^{12}C_i$ (5–10 μM) also entered the suspension. The cells were allowed to fix this small amount of $^{12}C_i$ before the addition of $^{13}C_i$ (noninhibited conditions of CO_2 fixation) or IAC (inhibited conditions of CO_2 fixation) to the medium. The C_i in the form of $K_2{}^{13}CO_3$ was added to a final concentration of 1 mM to ensure saturating concentrations of $^{13}C_i$ for photosynthesis and to minimize the interference that could occur because of refixation of respiratory $^{12}CO_2$ released into the medium during the course of the experiment. CA (carbonate dehydratase, EC 4.2.1.1) was also present in the suspension so that the CO_2 concentration was always directly proportional to the C_i concentration (Badger and Price, 1994). Rates of $^{13}C_i$ uptake were calculated from the slope of the m/z 45 signal. The values for U_0 and E_0 were obtained from the expressions given by Radmer and Kok (1976).

NO_2^- Analysis

Samples (50 μL) of cells with medium were taken at discrete intervals throughout the O_2 evolution measurements and immediately frozen in liquid nitrogen or killed in 80% alcohol. The NO_2^- content of the samples was determined according to the method of Strickland and Parsons (1968). NO_2^- reduction was measured as the disappearance of NO_2^- from the reaction mixture.

Chl Measurements

Chl was measured by extraction in methanol (Mackinney, 1941).

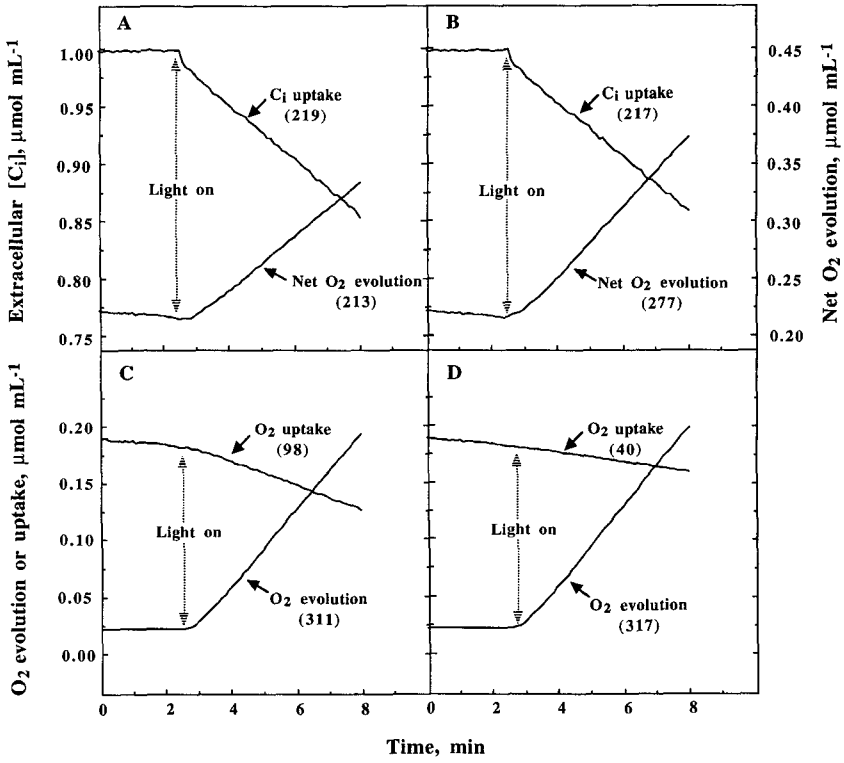

Figure 2. O_2 evolution and C_i uptake (A and B) and O_2 exchange (C and D) by *Synechococcus* UTEX 625 without (A and C) and with NO_2^- (B and D) under light conditions that are saturating for photosynthesis. The cells were preincubated in the light to remove C_i from the medium. The cell suspension was then flushed briefly with nitrogen to bring the $[^{16}O_2]$ to zero. A bubble of $^{18}O_2$ was introduced in the medium and left until the $[^{18}O_2]$ had reached about 190 μM. At this point the lights were turned off and then $K_2{}^{13}CO_3$ was added to a final concentration of 1 mM. The cells were dark adapted for 2.5 min and lights were turned on. NO_2^-, when present, was added in the dark to a final concentration of 5 mM. The $[^{16}O_2]$ and $[^{18}O_2]$ at the start of the experiment were 22 and 185 μM, respectively. Actinic light was 300 μmol m^{-2} s^{-1} (PAR), Chl *a* was 3 to 5 μg mL^{-1}, and 25 mM NaCl and 25 μg mL^{-1} CA were present in the reaction mixture. The values in parentheses for C_i uptake, O_2 evolution, and O_2 uptake are expressed in μmol mg^{-1} Chl h^{-1}. E_0 and U_0 were calculated essentially as described by Radmer and Kok (1976). Net O_2 evolution represents the difference between E_0 and U_0. The C_i uptake is based on ^{13}C measurements. Similar experiments were run at least three times. The E_0 in the light in the absence of C_i was 47 μmol mg^{-1} Chl h^{-1}, which was compensated by an equal amount of O_2 consumption.

Chemicals

CA and BTP were obtained from Sigma. $K_2{}^{13}CO_3$ (99 atom % ^{13}C) and $^{18}O_2$ (97.4 atom % ^{18}O) were obtained from MSD Isotopes (Montreal, Quebec, Canada).

RESULTS

Internal C_i Pool

The internal C_i pool size measurements were made with the mass spectrometer under both noninhibited and inhibited conditions of CO_2 fixation. Addition of 1 mM C_i to the reaction vessel resulted in an internal C_i pool of 39 mM (Fig. 1A) under noninhibited conditions of CO_2 fixation. The C_i pool size increased to 50 mM (Fig. 1B) when CO_2 fixation was blocked by IAC or glycolaldehyde. In general, the C_i pool within the cells was formed within about 60 s after the lights were turned on. The C_i pool within the cells reached its maximum size at a light intensity of 80 μmol m^{-2} s^{-1} and remained uninfluenced by any further increase in the light thereafter (data not shown). Addition of NO_2^- seemed to have no influence on the size of the internal C_i pool within the cells (Mir et al., 1995).

Effect of NO_2^- Assimilation on O_2 Exchange, Net O_2 Evolution, and Carbon Fixation

The effect of NO_2^- addition on isotopic O_2 exchange and simultaneous C_i fixation was studied under saturating (Fig. 2) and subsaturating (Fig. 3) light intensities for photosynthesis of *Synechococcus* UTEX 625. Simultaneous measurements of NO_2^- disappearance from the medium were also recorded. A rapid depletion of C_i from the medium was observed in the 1st min after the lights were turned on (Figs. 2, A and B, and 3, A and B), which was due to the formation of the C_i pool within the cells in addition to C_i fixation. When the C_i pool was filled to its maximum size, a steady-state rate of C_i depletion from the medium was maintained (Figs. 2, A and B, and 3, A and B). This C_i depletion resulted in almost equal net evolution of O_2 in the medium (Figs. 2A and 3A). When only CO_2 fixation was allowed, O_2 uptake represented 32% of E_0 (Fig. 2C) in

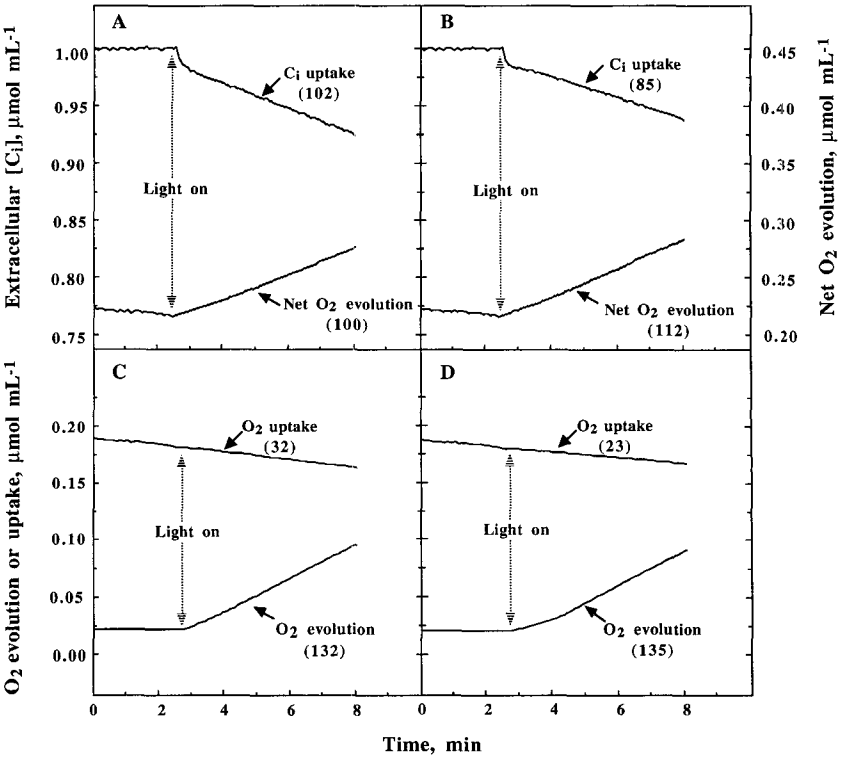

Figure 3. O_2 evolution and C_i uptake (A and B) and O_2 exchange (C and D) by *Synechococcus* UTEX 625 without (A and C) or with NO_2^- (B and D) under light conditions that are subsaturating for photosynthesis. All of the experimental details were the same as given in Figure 2 except that actinic light was 80 μmol m^{-2} s^{-1}. The values in parentheses for uptake and evolution are expressed in μmol mg^{-1} Chl h^{-1}.

high light. Whereas the addition of NO_2^- to the medium in high light increased the total electron capacity slightly as measured by E_0 (cf. C and D in Fig. 2), the O_2 consumption in the light was decreased tremendously (cf. C and D in Fig. 2). This decrease in O_2 uptake accounted largely for the observed increase in net O_2 evolution when NO_2^- assimilation and CO_2 fixation occurred together (Fig. 2B) compared to when only CO_2 assimilation occurred (Fig. 2A). The C_i uptake from the medium remained uninfluenced by NO_2^- assimilation at high light (cf. A and B, Fig. 2).

The data obtained under light-limited photosynthesis (Fig. 3) revealed that O_2 uptake represented only 25% of E_0 (Fig. 3C). The fraction of O_2 evolution that was not offset by O_2 uptake (net O_2 evolution) was compensated by an equal uptake of C_i from the medium (Fig. 3A). E_0 remained uninfluenced by NO_2^- addition (cf. C and D in Fig. 3), but both O_2 uptake (Fig. 3D) and C_i uptake (Fig. 3B) were reduced in contrast to only O_2 uptake in high light (Fig. 2, B and D).

NO_2^- Reduction

NO_2^- reduction rates of 36 (Fig. 2B) and 15 (Fig. 3B) μmol mg^{-1} Chl h^{-1} were recorded at saturating and subsaturating light intensities for photosynthesis. Net O_2 evolution, unaccounted for by C_i uptake, was 1.7 and 1.8 times the rate of NO_2^- reduction, respectively.

Effect of NO_2^- Assimilation on Net O_2 Evolution and Carbon Fixation at Various Light Intensities

The effect of NO_2^- assimilation on carbon fixation and concomitant O_2 evolution at various light intensities is presented in Figure 4. The O_2 evolution due to CO_2 fixation plateaued at a light intensity of 200 μmol m^{-2} s^{-1} (Fig. 4A). In general, O_2 evolution and carbon fixation maintained a ratio of 1:1 during photosynthesis at all light intensities (Fig. 4). These results are in agreement with those previously reported by Miller et al. (1988). NO_2^- assimilation increased the O_2 evolution at all light intensities, the effect being more discernible at high light (Fig. 4A). Although NO_2^- assimilation suppressed CO_2 fixation under light limitation, it had no effect on carbon fixation at high light intensities (Fig. 4B).

NO_2^- reduction, O_2 evolution that could be due to NO_2^- reduction, and the ratio of O_2 evolution due to NO_2^- reduction over NO_2^- reduction are presented in Table I. NO_2^- reduction increased with an increase in light intensity. Using a stochiometry of 1:1 between carbon fixation and O_2 evolution due to carbon fixation, we calculated the evolution of O_2 that could be due to NO_2^- reduction during simultaneous assimilation of carbon and nitrogen by *Synechococcus* UTEX 625 cells. The O_2 evolution due to NO_2^- reduction/NO_2^- reduction ratio was close to the expected ratio of 1.5 at each light intensity. The increased net O_2 evolution due to NO_2^- reduction at high light intensities (Fig. 4A) could then result only from a decrease in O_2 photoreduction.

Effect of NO_2^- Reduction on O_2 Exchange and Net O_2 Evolution under Inhibited Conditions of CO_2 Fixation

Light-dependent photoreduction of O_2 and NO_2^- in inhibited cells in the absence and presence of a C_i pool within the cells are presented in Table II. In the absence of a C_i pool, equal amounts of O_2 evolution and uptake of 47 μmol mg^{-1} Chl h^{-1} were obtained. NO_2^- assimilation in C_i-

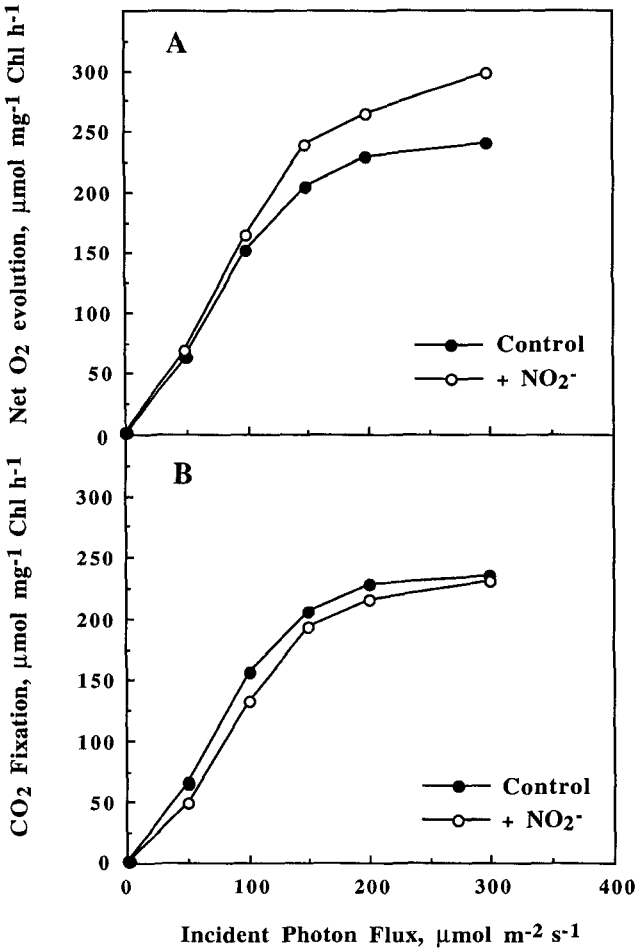

Figure 4. Effect of NO_2^- assimilation on net O_2 evolution (A) and CO_2 fixation (B) by *Synechococcus* UTEX 625 at various intensities of light. The cells were preincubated in the light to remove C_i from the medium. The lights were turned off and cells were dark adapted for 2.5 min. C_i in the form of $K_2{}^{13}CO_3$ was added to a final concentration of 1 mM in the dark. The lights were turned on and O_2 evolution and CO_2 fixation were monitored mass spectrometrically as described by Miller et al. (1988) at various light intensities. Parallel experiments were conducted, and the influence of NO_2^- assimilation on carbon fixation was determined at each light intensity. The NO_2^- was added in the dark to a final concentration of 5 mM. Chl *a* was 5 to 7 μg mL^{-1}, and 25 mM NaCl and 25 μg mL^{-1} CA were present in the reaction mixture.

depleted cells stimulated E_0 and suppressed O_2 uptake, and as a result, net O_2 evolution of 39 μmol mg^{-1} Chl h^{-1} was observed. When the cells were allowed to develop a C_i pool by addition of 1 mM C_i in the presence of IAC, the photoreduction of O_2 was stimulated 5-fold with no net O_2 evolution. Miller et al. (1988) also reported a lack of net O_2 evolution by the cells of *Synechococcus* UTEX 625 under inhibited conditions of CO_2 fixation. In the presence of a C_i pool when O_2 was the only electron acceptor, the electron transport as measured by E_0 was maintained at 78% (Table II) of the rate observed during C_i-saturated photosynthesis under similar light conditions (Fig. 2C). Addition of NO_2^- to the medium under inhibited conditions of CO_2 fixation but in the presence of C_i increased the electron transport (Table II) almost to the maximal capacity (Fig. 2C). In

Table I. *Effect of light intensity on NO_2^- reduction and O_2 evolution due to NO_2^- reduction during simultaneous assimilation of carbon and nitrogen by Synechococcus UTEX 625 in the light*

NO_2^- reduction was measured as the disappearance of NO_2^- from the medium of the cell suspensions described in Figure 4. Using a stochiometry of 1:1 between carbon fixation and O_2 evolution due to carbon fixation, we calculated the O_2 evolution that could be due to NO_2^- reduction at each light intensity. The O_2 concentration at the time of treatment initiation was 50 μM. The light intensity is given in μmol m^{-2} s^{-1}. The values are averages of two experiments and expressed in μmol mg^{-1} Chl h^{-1}.

Light Intensity	NO_2^- Reduction	O_2 Evolution	O_2 Evolution/ NO_2^- Reduction
50	14	22	1.6
100	22	38	1.7
150	26	48	1.8
200	30	47	1.5
300	36	62	1.7

contrast, NO_2^- addition suppressed O_2 uptake by 54% (Table II), and a net evolution of 176 μmol O_2 mg^{-1}Chl h^{-1} was observed. NO_2^- reduction measured as NO_2^- disappearance from the medium increased 4.5-fold in the presence of C_i (Table II). These results are in line with our previous results (Mir et al., 1995). O_2 evolution was 1.6 times the rate of NO_2^- reduction, which balances the electron supply from photosplitting of water with electron use in NO_2^- reduction.

DISCUSSION

Previous fluorescence studies indicated that O_2 (Miller et al., 1988, 1991; Canvin et al., 1990; Badger and Schreiber, 1993) and NO_2^- (Mir et al., 1995) can serve as efficient acceptors of electrons from the photosynthetic electron transport chain in *Synechococcus* UTEX 625 both when CO_2 fixation is allowed and when it is inhibited. An increase in O_2 photoreduction is observed when C_i is added and when CO_2 fixation is allowed (Fig. 2C), but the most dramatic increases in O_2 photoreduction (5-fold) or NO_2^- reduction (4.5-fold) are observed upon the addition of C_i when CO_2 fixation is inhibited (Table II).

NO_2^- is reduced via Fd (Miguel and Lara, 1987), and O_2 is photoreduced via either Fd or a component on the reducing side of PSI (Badger, 1985; Asada and Takahashi, 1987; Canvin et al., 1990). The stimulation of electron flow to O_2 and NO_2^- by C_i would then place the site of action of the C_i pool in the

Table II. *Effect of the C_i pool on O_2 and NO_2^- reduction under inhibited conditions of carbon fixation in Synechococcus UTEX 625*

The experimental details are as shown in Figure 2 except that IAC (3.3 mM) was added at the CO_2 compensation point. The values are averages of two experiments and are expressed in μmol mg^{-1} Chl h^{-1}.

Parameter	$-C_i$	$-C_i + NO_2^-$	$+C_i$	$+Ci + NO_2^-$
U_0	47	22	244	113
E_0	47	61	244	289
Net O_2 evolution	0	39	0	176
NO_2^- reduction	–	25	–	113
Net O_2/NO_2^- reduction	–	1.6	–	1.6

electron transport chain previous to Fd, and one might expect to observe competition among O_2, NO_2^-, and CO_2 for electrons from the photosynthetic electron transport chain.

At low light (at which electron transport is limiting), the addition of NO_2^- reduces both CO_2 fixation and O_2 photoreduction (Fig. 3). CO_2 fixation reduces O_2 photoreduction (cf. Table II and Fig. 2C). When CO_2 reduction is saturated (Fig. 4) or inhibited (Table II), the addition of NO_2^- reduces O_2 photoreduction (Fig. 2). These interactions would suggest that CO_2, O_2, and NO_2^- all compete for electrons at a similar site. They might also suggest that the effect of internal C_i on photosynthetic electron flow is at a position common to the reduction of all three substrates (Mir et al., 1995) rather than at a subsequent position involving the reduction of only one substrate (e.g. O_2) (Badger and Schreiber, 1993).

Any one substrate (i.e. CO_2 or O_2 or NO_2^-), however, does not seem able to accept all of the electrons from the photosynthetic electron transport chain or completely suppress the reduction of the others. At high light (at which electron transport is not limiting), the photoreduction of O_2 becomes additive to the maximum rate of CO_2 fixation. This is not normally observed, because the additional photoreduction of O_2 with increased light intensity is balanced by additional O_2 evolution, and net O_2 evolution, which is equal to CO_2 fixation, is unaffected (Fig. 4). However, when NO_2^- is added, an increase in net O_2 evolution is observed (Figs. 2–4) with no increase in E_0 (Fig. 2, C and D). This must mean that the increase in net O_2 evolution upon addition of NO_2^- (Fig. 4) is a direct measure of the suppression of O_2 photoreduction (Fig. 2). An increase in net O_2 evolution upon addition of NO_3^- to high-CO_2-grown cells of *Anacystis nidulans* was also reported by Romero and Lara (1987). When O_2 photoreduction is saturated the addition of NO_2^- results in an increase in E_0 (Table II). This would suggest that the reducing capacity for any one substrate was less than the capacity of the photosynthetic electron transport chain. Competition was observed when the electron supply was limiting, but additivity was observed when the electron supply was not limiting.

To determine the mechanism by which the C_i pool stimulates photosynthetic electron flow it is necessary to know the site at which it acts. In previous papers (Miller et al., 1988; Canvin et al., 1990) it was suggested that C_i affected some component of the electron transport chain, but studies with artificial electron acceptors for PSI and PSII failed to show any effect of the C_i pool on the reduction of these acceptors (Badger and Schreiber, 1993), and it was suggested that the "C_i pool can directly stimulate the ability of O_2 to act as a PSI acceptor." If this is so, then it must also directly stimulate NO_2^- reduction, but as far as is known NO_2^- reductase is not affected by C_i. The apparent competition among the reduction reactions of O_2, NO_2^-, and CO_2 when the electron supply was limited (low light) would indicate that the increased electron flow was available to all three substrates. The observation of increased electron flow upon the addition of another substrate when electron supply was not limiting (high light) would also suggest that C_i had relieved some impediment in the electron transport chain. These results place the site of action of the C_i pool on a component of the electron transport chain and suggest that the reduction of artificial electron acceptors should be reinvestigated under conditions under which the C_i status of the reaction mixture is known from direct measurements.

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