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Photosynthetic Nitrite Reduction as Influenced by the Internal Inorganic Carbon Pool in Air-Grown Cells of *Synechococcus* UTEX 625[1]

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Photosynthetic reduction of NO_2^- was studied in air-grown cells of a cyanobacterium, *Synechococcus* UTEX 625. Addition of NO_2^- resulted in significant amounts of chlorophyll *a* fluorescence quenching both in the absence and presence of CO_2 fixation inhibitors, glycolaldehyde or iodoacetamide. The degree of NO_2^- quenching was insensitive to the O_2 concentration in the medium. Addition of 100 μM inorganic carbon in the presence of glycolaldehyde and O_2, leading to formation of the carbon pool within the cells, resulted in pronounced fluorescence quenching. Removal of O_2 from the medium restored the fluorescence yield completely, and the subsequent addition of NO_2^- quenched 36% of the variable fluorescence. From the response to added 3-(3,4-dichlorophenyl)-1,1-dimethylurea, the quenching by NO_2^- appeared to be photochemical quenching, and nonphotochemical quenching did not seem to be present. The reduction of NO_2^- observed on its addition to inorganic carbon-depleted cells remained uninfluenced by O_2 or glycolaldehyde. The internal inorganic carbon pool in the cells stimulated NO_2^- reduction, both in the presence and absence of O_2, by 4.8-fold. An increase in NO_2^- reduction by 0.5-fold was also observed in the presence of O_2 during simultaneous assimilation of carbon and nitrogen in inorganic carbon-depleted cells. Contrary to this, under anaerobiosis, NO_2^- reduction was suppressed when carbon and nitrogen assimilation occurred together.

Photosynthetic electron use by CO_2 reduction results in significant amounts of Chl *a* fluorescence quenching in *Synechococcus* (Miller et al., 1991; Badger and Schreiber, 1993). Fluorescence quenching on addition of C_i to the cells was similar, however, when CO_2 fixation was inhibited by GLY or IAC. Removal of O_2 from the medium restored the fluorescence yield to its original level, indicating that O_2 was functioning as an electron acceptor under the conditions of inhibited CO_2 fixation. Mass spectrometric measurements revealed that a large internal C_i pool developed when CO_2 fixation in the presence of light was inhibited (Canvin et al., 1990). This accumulated C_i pool in some way accelerated O_2 photoreduction and led to fluorescence quenching. O_2 appears to be reduced by Fd or the reducing side of PSI (Badger, 1985; Asada and Takahashi, 1987; Canvin et al., 1990), and it is possible that the energy required for maintaining the C_i pool may result from pseudocyclic photophosphorylation during O_2 photoreduction (Sültemeyer et al., 1993).

The assimilation of inorganic forms of nitrogen, NO_3^- or NO_2^-, consumes photosynthetically generated assimilatory power in blue-green algae (Serrano et al., 1981, 1982; Romero and Lara, 1987). That NO_2^- accepts electrons from Fd in green cells is unequivocally accepted (Miguel and Lara, 1987). In *Synechococcus* UTEX 625, the flow of electrons from the photosplitting of water to Fd appears to be influenced by the internal C_i pool (Miller et al., 1991) as evidenced by the enhanced O_2 photoreduction.

In this paper, we examine the reduction of NO_2^- by *Synechococcus* UTEX 625 in the light and the effect of the internal C_i pool on the rate of reduction.

MATERIALS AND METHODS

The unicellular cyanobacterium *Synechococcus leopoliensis* UTEX 625 (University of Texas Culture Collection, Austin, TX) 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 $NaNO_3$.

Experimental Conditions

Cells were washed three times by centrifugation (1 min at 10,000*g*, Beckman Microfuge B) and resuspended (10–15 μg Chl mL^{-1}) in 25 mM 1,3-bis[tris(hydroxymethyl)-methylamino]propane-HCl buffer, pH 8.0. This buffer contains only 10 to 20 μM C_i when it is kept under N_2 in a stoppered serum 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. Two milliliters (O_2 electrode) or 6 mL (mass spectrometer) of cell suspension were transferred to the reaction chamber and allowed to reach the CO_2 compensation point after the addition of 25 mM NaCl. Illumination was provided by a tungsten halogen projection lamp. NO_2^- was added as KNO_2.

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Abbreviations: C_i, dissolved inorganic carbon (CO_2 plus HCO_3^- plus CO_3^{2-}); F_m*, maximum fluorescence in the absence of inorganic carbon; F_v, variable fluorescence, the difference between F_m* and minimal fluorescence (dark); GLY, glycolaldehyde; IAC, iodoacetamide; QA, primary electron accepting plastoquinone of PSII.

Measurement of Chl *a* Fluorescence Quenching

Fluorescence yield was measured with a pulse modulation fluorometer (PAM-101, H. Walz, Effeltrich, Germany). Actinic light was 60 μmol m^{-2} s^{-1}. The pulse-modulated measuring beam (100 kHz) was 1 μmol m^{-2} s^{-1}. The oxidation state of Q_A was routinely measured at 60-s intervals with a 1-s flash of high-intensity white light of 1600 μmol m^{-2} s^{-1}. The results were recorded with a Linear recorder. The influence of internally accumulated C_i on photosynthetic electron transport and fluorescence yield was also determined using artificial electron acceptors/inhibitors (Badger and Schreiber, 1993). The fluorescence terminology used in this paper conforms as closely as possible to the recommended standard nomenclature (van Kooten and Snell, 1990) with a few additional terms coined by Miller et al. (1991).

Measurement of O_2 Evolution

O_2 evolution was determined from cell suspensions at a light intensity of 300 μmol m^{-2} s^{-1} using a Hansatech (King's Lynn, Norfolk, UK) DW 2 O_2 electrode as described by Miller and Canvin (1987).

NO_2^- and Chl Measurement Analysis

Samples (50 μL) of cells with medium were obtained 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 (1972). NO_2^- reduction was measured as the disappearance of NO_2^- from the reaction mixture. Chl was measured by extraction in methanol (Meeks et al., 1983).

Chemicals

Carbonic anhydrase (carbonate dehydratase, EC 4.2.1.1) and 1,3-bis[tris(hydroxymethyl)-methylamino]propane were obtained from Sigma Chemical Co. $K_2{}^{13}CO_3$ (99 atom % ^{13}C) was obtained from MSD Isotopes (Montreal, Canada).

RESULTS

Effect of External NO_2^- Concentration on NO_2^- Reduction and O_2 Evolution

Light-dependent NO_2^- reduction and O_2 evolution by *Synechococcus* UTEX 625 are presented in Table I. NO_2^- reduction and O_2 evolution increased up to 10 mM; the increase was linear up to 2 mM NO_2^- in the medium. Because the maximum rate of reduction occurred at 10 mM NO_2^-, this concentration was used in subsequent experiments. It is not known why a lower rate of NO_2^- reduction was observed at 20 mM NO_2^-. The O_2 evolution/NO_2^- reduction ratio (Table I) was always higher than the expected ratio of 1.5.

Table I. *Effect of NO_2^- concentration on O_2 evolution and NO_2^- reduction by Synechococcus UTEX 625 in the light*

NO_2^- reduction was measured as the disappearance of NO_2^- from the medium. Cells depleted of C_i were placed in the O_2 electrode (30°C), and O_2 evolution was initiated by the addition of NO_2^-. The light intensity was 300 μmol m^{-2}s^{-1}, and O_2 concentration at the time of treatment initiation was 50 μM. The values are averages of two experiments, which agreed in most treatments within 10% and are expressed in μmol mg^{-1} Chl h^{-1}. NO_2^- reduction in the dark was 2 μmol mg^{-1} Chl h^{-1}.

[NO_2^-]	O_2 Evolution	NO_2^- Reduction	O_2 Evolution/ NO_2^- Reduction
mM			
1	10	5.4	1.8
2	20	12.2	1.6
5	29	16.7	1.7
10	39	23.8	1.6
20	12	5.5	2.1

Internal C_i Pool

Internal C_i pool size measurements were made with the mass spectrometer (Miller et al., 1988) in both the presence and absence of O_2. Addition of 100 μM C_i led to formation of an internal C_i pool of 35 mM in the presence of O_2. The formation of the C_i pool stimulates O_2 photoreduction (Miller et al., 1991), and it was proposed that the energy required for maintaining this C_i pool may result from pseudocyclic phosphorylation (Sültemeyer et al., 1993). In that case, one would expect leakage of the C_i pool under inhibited conditions of CO_2 fixation when O_2 was removed from the medium. It is interesting that the C_i pool size increased to 52 mM when O_2 was removed from the medium. It is possible that during anaerobiosis and under inhibited conditions of CO_2 fixation the energy required for C_i pool formation may result from cyclic phosphorylation alone, and that could explain the increase in C_i pool size observed when O_2 was removed from the medium. The addition of NO_2^- also seemed to have no influence on the size of the internal C_i pool.

Chl *a* Fluorescence

Figure 1 depicts the effect of added NO_2^- on Chl *a* fluorescence in air-grown *Synechococcus* UTEX 625. The F_m* was observed in the absence of inorganic carbon, evident by the lack of net O_2 evolution. The illumination of the cells with a saturating flash of light in the absence of C_i (start of each trace) increased the fluorescence yield by only a small amount (Fig. 1), indicating that Q_A was largely reduced (Schreiber et al., 1986). The addition of NO_2^- resulted in a pronounced quenching of fluorescence, reaching a value of 30% of F_v. However, this maximum decrease in fluorescence yield was sustained for only 2 to 3 min and stabilized thereafter at 17% of F_v. From the quenching pattern observed on addition of NO_2^-, one could speculate that the initial maximum quenching could be due to small amounts of C_i in the solution. In that case, one could attribute it to CO_2 fixation apart from NO_2^- reduction.

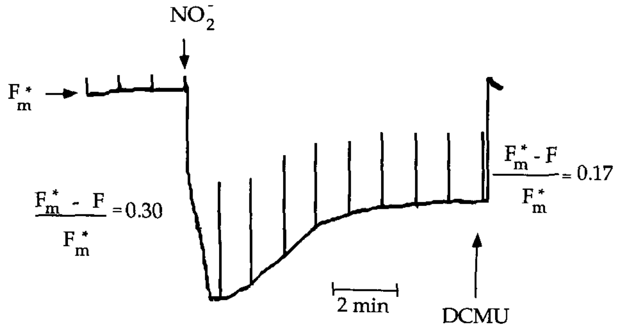

Figure 1. Effect of NO_2^- on Chl *a* fluorescence (*F*) of *Synechococcus* UTEX 625. The cells were preincubated in the light to remove C_i from the medium, and the reaction was started by the addition of 10 mM NO_2^-. Actinic light was 60 μmol m^{-2} s^{-1} (PAR), Chl *a* was 9 to 10 μg mL^{-1}, and 25 mM NaCl was present in the reaction mixture. DCMU addition was 20 μM.

One way to test this hypothesis was to stop CO_2 fixation by the addition of a CO_2 fixation inhibitor, GLY or IAC. If the initial maximum quenching was due to small amounts of C_i in the NO_2^- solution, then inhibition of CO_2 reduction would allow the cells to maintain the initial maximum quenching level until the NO_2^- was completely reduced. However, addition of 12 mM GLY or 3.3 mM IAC to the medium (data not shown) had no effect on the overall quenching pattern due to NO_2^- addition. Also, when the NO_2^- for a 10 mM final concentration was added in one-tenth of the volume (thereby changing the amount of C_i that could be added), there was no effect on the fluorescence. In *Synechococcus* UTEX 625, CO_2 does not cause any quenching of fluorescence under conditions of inhibited CO_2 fixation in the absence of O_2 (Miller et al., 1991). The experiment as shown in Figure 1 was repeated at zero $[O_2]$ in the presence of the CO_2 fixation inhibitor, GLY or IAC, to further confirm the pattern of NO_2^--induced quenching. The amount and degree of Chl *a* fluorescence quenching remained uninfluenced by the O_2 content in the medium (data not shown). Most of the quenching induced by addition of NO_2^- was transiently relieved when cells were illuminated with a saturating flash of light. This demonstrated that much of the quenching caused by addition of NO_2^- was due to increased oxidation of Q_A.

Since only a proportion of NO_2^--induced quenching was relieved when the cells were illuminated with a saturating flash of light, one could think that the portion of quenching not relieved during a saturating flash of light might be energy-dependent quenching in *Synechococcus* UTEX 625. However, the estimation of photochemical quenching due to Q_A oxidation in this organism was higher when measured by DCMU addition than when measured by illuminating the cells with a saturating flash of light (Fig. 1). Addition of DCMU indicated that almost 100% of the quenching was due to Q_A oxidation, whereas only 60%, on average, of the quenching was relieved during a saturating flash of light. The intensity of the light flash was fully saturating with respect to PSII absorption (data not shown).

Figure 2 shows that addition of C_i initiated the quenching of Chl *a* fluorescence under conditions of inhibited CO_2 fixation. It has already been shown that electrons that pass through PSII are ultimately accepted by O_2 when their use for CO_2 reduction is blocked (Miller et al., 1991). Addition of NO_2^- caused an additional quenching of about 9% of F_v. This would mean that NO_2^- efficiently competes for electrons with O_2, but the relative contribution of O_2 or NO_2^- as electron acceptor toward overall quenching of Chl *a* fluorescence under these conditions is not known.

The addition of C_i also caused quenching of Chl *a* fluorescence (Fig. 3) when the O_2 was removed from the medium by addition of the Glc oxidase system but CO_2 fixation was allowed. Most of the quenching was transiently relieved during a saturating flash of light, indicating that the addition of C_i resulted in an enhanced rate of Q_A oxidation. The kinetics of Q_A reoxidation when only CO_2 could act as an electron acceptor were slow, requiring 40 to 45 s for oxidation to the original level. These results are in agreement with those reported by Miller et al. (1991). Addition of NO_2^- resulted in a further quenching of Chl *a* fluorescence by 9% in F_v. A large proportion of the quenching was transiently relieved with each saturating flash of light provided, demonstrating that it was photochemical quenching. With NO_2^- as an electron acceptor, the kinetics of Q_A reoxidation were changed and reoxidation was much faster.

Figure 4 demonstrates that the addition of C_i in the presence of GLY and anaerobic conditions did not cause any quenching of Chl *a* fluorescence. The fluorescence yield was close to the maximum (F_m*) under conditions of inhibited CO_2 fixation. PSII fluorescence quenching in *Synechococcus* as a result of electron transport approaches zero at zero $[O_2]$ (Miller et al., 1991; Badger and Schreiber, 1993). Under these conditions, however, the addition of NO_2^- resulted in 36% of F_v quenching. The peaks on each satu-

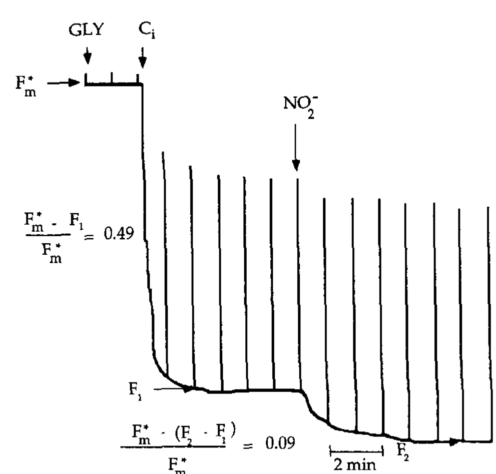

Figure 2. Effect of O_2 and NO_2^- on F_v of *Synechococcus* UTEX 625 in the presence of GLY (12 mM) and C_i. GLY was added to the C_i-depleted cells, followed by the addition of 100 μM C_i and 10 mM NO_2^- as shown. O_2 concentration was 240 μM. Actinic light was 60 μmol m^{-2} s^{-1} (PAR), Chl *a* was 9 to 10 μg mL^{-1}, and 25 mM NaCl was present in the reaction mixture.

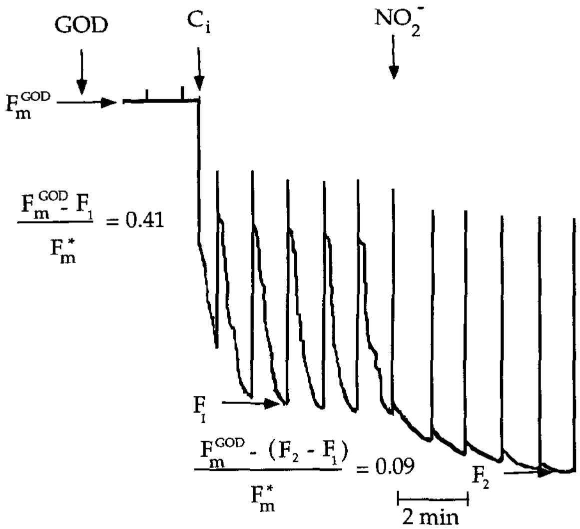

Figure 3. Effect of CO_2 and NO_2^- as electron acceptors on F_v of *Synechococcus* UTEX 625 in the absence of O_2. Cells were depleted of C_i, and O_2 was removed from the medium by addition of the Glc oxidase system (GOD), which consisted of 10 mM Glc, 100 μg mL^{-1} Glc oxidase, and 50 μg mL^{-1} catalase. The GOD system was added 12 min before C_i and the fluorescence pattern upon addition of GOD is not shown. This pattern may be seen in Figure 4, where the addition of GOD results in substantial quenching of F_v, which is gradually relieved. The observed initial quenching is caused by C_i in solutions of the GOD system and quenching is relieved as this C_i is fixed. After addition of the GOD system, however, the fluorescence does not return to F_m* but to a slightly lower level (Fig. 4) designated F_m^{GOD}. Quenching of F_v was calculated using F_m^{GOD} as the maximum fluorescence under these conditions. The reaction was started by addition of 100 μM C_i, followed by 10 mM NO_2^-.

ration flash of light were sharp, and reduction and reoxidation of Q_A were rapid. When DCMU (20 μM) was added to the medium, the fluorescence yield rapidly returned to the original level, further indicating that the quenching was photochemical (Krause et al., 1982).

NO_2^- Assimilation and O_2 Evolution

The NO_2^- assimilation and O_2 evolution data obtained in various treatments are presented in Table II. The addition of NO_2^- to C_i-depleted cells resulted in NO_2^- assimilation rates of 26 μmol mg^{-1} Chl h^{-1}. Removal of O_2 or addition of GLY to the medium did not influence the NO_2^- assimilation rates. Miller and Canvin (1987) and Miller et al. (1991) reported that the internal inorganic carbon pool stimulated the flow of electrons from water to Fd, resulting in O_2 photoreduction. Since NO_2^- also accepts electrons from Fd, we tested this hypothesis using NO_2^- as a terminal electron acceptor. When the cells were allowed to build up an inorganic carbon pool by addition of 100 μM C_i in the presence of GLY, the O_2 evolution rate as a consequence of NO_2^- reduction was more than 70% of the rate of photosynthesis. Removal of O_2 from the medium under similar conditions resulted in an additional 18% increase in NO_2^- reduction. NO_2^- reduction was also influenced by the internal C_i pool when CO_2 fixation and NO_2^- assimilation were allowed simultaneously. NO_2^- assimilation in the presence of O_2 was increased by 53% but was suppressed by 37% when O_2 was removed from the medium.

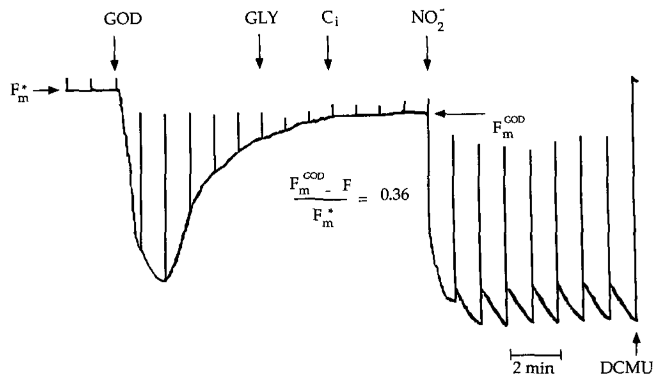

Figure 4. Effect of NO_2^- as electron acceptor on F_v of *Synechococcus* UTEX 625 inhibited with GLY (12 mM) in the absence of O_2. Cells were depleted of C_i, O_2 was removed from the medium, and 100 μM C_i and 10 mM NO_2^- were added as shown. DCMU addition was 20 μM. The addition of the Glc oxidase system (GOD) caused some quenching and the establishment of a level of fluorescence (*F*) lower than F_m*. This level is identified as F_m^{GOD}, and the quenching of F_v from the addition of NO_2^- is calculated using that level as maximum fluorescence under these conditions.

In general, the O_2 evolution (Tables I and II) was 1.5 to 1.8 times the rate of NO_2^- reduction, which balances the electron supply from photosplitting of water with electron use in NO_2^- reduction.

DISCUSSION

In the absence of inorganic carbon, cyanobacteria such as *Synechococcus* display maximum Chl *a* fluorescence with no apparent nonphotochemical quenching. Fluorescence can

Table II. *Effect of the internal inorganic carbon pool on O_2 evolution and NO_2^- reduction by Synechococcus UTEX 625 in the light*

Cells depleted of C_i were placed in O_2 electrode (30°C), and treatments were initiated. NO_2^- was added to a final concentration of 10 mM, and GLY and C_i were added to concentrations of 12 mM and 100 μm, respectively. O_2, when present, was 50 μM at the time of treatment initiation. When required, anaerobiosis was achieved by adding Glc oxidase (final concentration, 100 μg mL^{-1}) to a cell suspension containing 10 mM Glc and 50 μg mL^{-1} catalase. O_2 evolution and NO_2^- reduction were measured and expressed in μmol mg^{-1} Chl h^{-1}. Light intensity was 300 μmol m^{-2}s^{-1}. The values are averages of two experiments, which agreed in most treatments within 10%. CO_2 reduction on addition of 100 μM C_i in control cells was 242 ± 10 μmol mg^{-1} Chl h^{-1}. –, Not determined.

Treatment	O_2 Evolution	NO_2^- Reduction	O_2 Evolution/NO_2^- Reduction
O_2	38	26	1.5
$-O_2$	–	26	–
O_2 + GLY	40	25	1.6
$-O_2$ + GLY	–	29	–
O_2 + GLY + C_i	174	120	1.5
$-O_2$ + GLY + C_i	–	141	–
O_2 + C_i	300	40	–
$-O_2$ + C_i	–	16	–

be quenched by the fixation of CO_2, but when CO_2 fixation is inhibited it can also be quenched by O_2 when an internal C_i pool is allowed to develop. The development of the internal C_i pool in some way not yet understood initiates the photoreduction of O_2 (Miller et al., 1991; Badger and Schreiber, 1993). Photoreduction appears to be via reduced Fd (Canvin et al., 1990).

NO_2^- and nitrate are reduced in cyanobacteria using reduced Fd as the source of electrons (Miguel and Lara, 1987), and they also cause fluorescence quenching (Serrano et al., 1981, 1982). One might expect then that the reduction of these compounds would also be influenced by the internal C_i pool.

NO_2^- addition in the absence of CO_2 did cause quenching (Fig. 1), and from the saturating flash results, at least 60% of the quenching could be attributed to photochemical quenching. Reduction and reoxidation of Q_A were rapid, similar to that observed when O_2 acted as the oxidant (Fig. 2). When O_2 acts as the oxidant, about 70 to 80% of the quenching is photochemical quenching (Fig. 2). As observed with O_2 quenching (Miller et al., 1991), increasing the intensity of the flash or diluting the cell concentration had no effect on the photochemical quenching due to NO_2^-. The fluorescence that is not recovered during a saturating flash may be appropriately assigned to nonphotochemical quenching (Schreiber and Neubauer, 1990), but the results with DCMU (Fig. 1) give no indication for two quenching components (Schreiber et al., 1986).

With either O_2 quenching or CO_2 quenching of fluorescence, the addition of NO_2^- caused an increase in quenching (Figs. 2 and 3). In addition, similar to the effect of O_2 (Miller et al., 1991), the slow reoxidation of Q_A by CO_2 was hastened by the addition of NO_2^- (Fig. 3). Thus, NO_2^- appears to be as efficient an oxidant as O_2, with both being much better than CO_2 alone.

In the absence of O_2 but in the presence of an internal C_i pool (Fig. 4), NO_2^- addition resulted in a fluorescence quenching equivalent to that observed with O_2 or CO_2 as the quenching agent. In this case, 70 to 80% of the quenching could be relieved by a saturating light flash, and DCMU addition indicated that all of the quenching could be attributed to photochemical quenching. The discrepancy between the saturating light flash results and the DCMU results in reference to photochemical quenching remains unexplainable.

As recorded previously (Flores et al., 1983), NO_2^- was reduced by the photosynthetic system, and in the absence of other electron acceptors the theoretical stoichiometry of 1.5 between O_2 evolution and NO_2^- reduction was observed (Table II). Although the absence or presence of O_2 or GLY had little effect on the rate of NO_2^- reduction, the formation of the internal C_i pool stimulated NO_2^- reduction 4-fold (Table II). NO_2^- reduction increased when O_2 was removed from this reaction mixture, suggesting that O_2 was perhaps competing for electrons. When carbon fixation was allowed, NO_2^- reduction was reduced, and the ratio of carbon assimilated to NO_2^- reduction was 6.5, a value of carbon to nitrogen assimilation that has been observed in *Scenedesmus* (Larsson et al., 1985).

The mechanism by which the C_i pool stimulates photosynthetic electron flow or the reduction of O_2 and NO_2^- remains unknown. It has been known for some time that HCO_3^- is required for the transfer of electrons from Q_A^- to plastoquinone (Cao and Govindjee, 1988), and the stimulation of electron flow in PSII of cyanobacteria by CO_2 or HCO_3^- has been observed (Vennesland et al., 1965; Cao and Govindjee, 1988). This possible mechanism for the HCO_3^- (C_i pool)-stimulated electron flow in PSII was discussed in our previous paper (Miller et al., 1991), and we pointed out that, whereas the K_d (HCO_3^-) for this effect was 35 to 60 μM in thylakoids isolated from spinach chloroplasts (Blubaugh and Govindjee, 1988), the electron flow observed in *Synechococcus* (as interpreted from fluorescence quenching) seemed to increase with concentrations of C_i increasing to 30 to 60 mM (Miller et al., 1991). On that basis it seemed unlikely that the HCO_3^- accumulated in cyanobacteria was exerting a direct effect on electron flow from Q_A^- to plastoquinone, and with the use of artificial electron acceptors, Badger and Schreiber (1993) recently showed that in the absence of C_i there did not appear to be any inhibition of any specific partial reactions in the photosynthetic electron transport chain. We have also used artificial electron acceptors to drain electrons at PSII (2,6-dimethylbenzoquinone plus ferricyanide [500 plus 500 μM] and 2,5-dibromo-3-methyl-6-isopropyl-*p*-benzoquinone [50 μM]) and PSI (methyl viologen [1 mM] and *N,N*-dimethyl-*p*-nitrosoaniline [200 μM]) from the electron transport chain in C_i-depleted cells. The rate of electron flow from water to these acceptors, as measured by O_2 evolution and fluorescence quenching, was maximal upon the addition of the acceptor. No stimulatory effect of C_i on electron flow to these acceptors was observed.

With the mass spectrometer, we also determined the effect of these artificial electron acceptors on the internal C_i pool. If the pool had been formed in GLY-inhibited cells, the pool instantly leaked out upon the addition of the acceptor. When the artificial electron acceptors were added to GLY-inhibited cells prior to C_i, no internal C_i pool developed within the cells. Hence, the C_i pool seemed to be nonessential for electron flow to these artificial electron transport acceptors, and C_i would appear to have no direct effect on the reactions of the electron transport chain. Badger and Schreiber (1993), in a more extensive investigation of electron transport, also came to that conclusion and suggested that the C_i pool stimulated the reduction of O_2 by PSI. Our results show that NO_2^- reduction is stimulated in a manner similar to O_2 photoreduction, and although we do not know the action of C_i, it is apparent that it must act similarly for NO_2^- reduction and O_2 photoreduction.

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LITERATURE CITED

Asada K, Takahashi M (1987) Production and scavenging of active oxygen in photosynthesis. *In* DJ Kyle, CB Osmond, CJ Arntzen, eds, Photoinhibition. Elsevier Science Publishers, Amsterdam, The Netherlands, pp 227–287

Badger MR (1985) Photosynthetic oxygen exchange. Annu Rev Plant Physiol **36:** 27–53

Badger MR, Schreiber U (1993) Effects of inorganic carbon accumulation on photosynthetic oxygen reduction and cyclic electron flow in the cyanobacterium *Synechococcus* PCC 7942. Photosynth Res **37:** 177–191

Blubaugh DJ, Govindjee (1988) The molecular mechanism of the bicarbonate effect at the plastoquinone reduction site of photosynthesis. Photosynth Res **19:** 85–128

Canvin DT, Miller AG, Espie GS (1990) Inorganic carbon concentrating processes in cyanobacteria. *In* SK Sinha, PV Sane, SC Bhargava, PK Agrawal, eds, Proceedings of the International Congress of Plant Physiology, Vol 1. Indian Agricultural Research Institute, New Delhi, India, pp 569–580

Cao J, Govindjee (1988) Bicarbonate effect on electron flow in a cyanobacterium *Synechococcus* PCC6803. Photosynth Res **19:** 277–285

Espie GS, Canvin DT (1987) Evidence for Na^+-independent HCO_3^- uptake by the cyanobacterium *Synechococcus leopoliensis*. Plant Physiol **84:** 125–130

Flores E, Guerrero MG, Losado M (1983) Photosynthetic nature of nitrate uptake and reduction in the cyanobacterium *Anacystis nidulans*. Biochim Biophys Acta **722:** 408–416

Krause GH, Vernotte C, Briantais JM (1982) Photoinduced quenching of chlorophyll fluorescence in intact chloroplasts and algae. Resolution into two components. Biochim Biophys Acta **679:** 116–124

Larsson M, Olsson T, Larsson CM (1985) Distribution of reducing power between photosynthetic carbon and nitrogen assimilation in *Scenedesmus*. Planta **164:** 246–253

Meeks JC, Wycoff KL, Chapman JS, Enderlin GS (1983) Regulation of expression of nitrate and dinitrogen assimilation by *Anabaena species*. Appl Environ Microbiol **45:** 1351–1359

Miguel GG, Lara C (1987) Assimilation of inorganic nitrogen. *In* P Fay, C Van Baalen, eds, The Cyanobacteria. Elsevier Science Publishers, Amsterdam, The Netherlands, pp 163–186

Miller AG, Canvin DT (1987) The quenching of chlorophyll *a* fluorescence as a consequence of the transport of inorganic carbon by the cyanobacterium *Synechoccoccus* UTEX 625. Biochim Biophys Acta **894:** 407–413

Miller AG, Espie GS, Canvin DT (1988) Active transport of CO_2 by the cyanobacterium *Synechoccoccus* UTEX 625. Measurement by mass spectrometry. Plant Physiol **86:** 677–683

Miller AG, Espie GS, Canvin DT (1991) The effects of inorganic carbon and oxygen on fluorescence in the cyanobacterium *Synechococcus* UTEX 625. Can J Bot **69:** 1151–1160

Miller AG, Turpin DH, Canvin DT (1984) Growth and photosynthesis of the cyanobacterium *Synechococcus leopoliensis* in HCO_3^--limited chemostats. Plant Physiol **75:** 1064–1070

Romero JM, Lara C (1987) Photosynthetic assimilation of NO_3^- by intact cells of the cyanobacterium *Anacystis nidulans*. I. Influence of NO_3^- and NH_4^+ assimilation on CO_2 fixation. Plant Physiol **83:** 208–212

Schreiber U, Neubauer C (1990) O_2 dependent electron flow, membrane energization and the mechanism of nonphotochemical quenching of chlorophyll fluorescence. Photosynth Res **25:** 279–293

Schreiber U, Schliwa U, Bilger W (1986) Continuous recording of photochemical and nonphotochemical chlorophyll fluorescence quenching with new modulation fluorometer. Photosynth Res **10:** 51–62

Serrano A, Rivas J, Losada M (1981) Nitrate and nitrite as in vivo quenchers of chlorophyll fluorescence in blue green algae. Photosynth Res **2:** 175–181

Serrano A, Rivas J, Losada M (1982) Changes in fluorescence spectra by nitrate and nitrite in blue green algae. Photobiochem Photobiophys **4:** 257–264

Strickland JDH, Parsons TR (1972) A practical handbook of sea water analysis. Fish Res Board Can Bull **167:** 71–89

Sültemeyer D, Biehler K, Fock HP (1993) Evidence for the contribution of pseudocyclic photophosphorylation to the energy requirement of the mechanism for concentrating inorganic carbon in *Chlamydomonas*. Planta **189:** 235–242

van Kooten O, Snell JFH (1990) The use of chlorophyll fluorescence nomenclature in plant stress physiology. Photosynth Res **25:** 147–150

Vennesland B, Olson E, Ammeraal RN (1965) Role of carbon dioxide in the hill reaction. Fed Proc **24:** 873–880