Plant Physiol. (1989) 89, 409–415
0032-0889/89/89/0409/07/$01.00/0

Received for publication June 7, 1988
and in revised form September 6, 1988

Mitochondrial Respiration Can Support NO_3^- and NO_2^- Reduction during Photosynthesis[1]

Interactions between Photosynthesis, Respiration, and N Assimilation in the N-Limited Green Alga *Selenastrum minutum*

Harold G. Weger and David H. Turpin*

Department of Biology, Queen's University, Kingston, Ontario, Canada K7L 3N6

ABSTRACT

Mass spectrometric analysis shows that assimilation of inorganic nitrogen (NH_4^+, NO_2^-, NO_3^-) by N-limited cells of *Selenastrum minutum* (Naeg.) Collins results in a stimulation of tricarboxylic acid cycle (TCA cycle) CO_2 release in both the light and dark. In a previous study we have shown that TCA cycle reductant generated during NH_4^+ assimilation is oxidized via the cytochrome electron transport chain, resulting in an increase in respiratory O_2 consumption during photosynthesis (HG Weger, DG Birch, IR Elrifi, DH Turpin [1988] Plant Physiol 86: 688–692). NO_3^- and NO_2^- assimilation resulted in a larger stimulation of TCA cycle CO_2 release than did NH_4^+, but a much smaller stimulation of mitochondrial O_2 consumption. NH_4^+ assimilation was the same in the light and dark and insensitive to DCMU, but was 82% inhibited by anaerobiosis in both the light and dark. NO_3^- and NO_2^- assimilation rates were maximal in the light, but assimilation could proceed at substantial rates in the light in the presence of DCMU and in the dark. Unlike NH_4^+, NO_3^- and NO_2^- assimilation were relatively insensitive to anaerobiosis. These results indicated that operation of the mitochondrial electron transport chain was not required to maintain TCA cycle activity during NO_3^- and NO_2^- assimilation, suggesting an alternative sink for TCA cycle generated reductant. Evaluation of changes in gross O_2 consumption during NO_3^- and NO_2^- assimilation suggest that TCA cycle reductant was exported to the chloroplast during photosynthesis and used to support NO_3^- and NO_2^- reduction.

Mitochondrial respiration consists of two interrelated processes: TCA[2] cycle carbon flow and mitochondrial electron transport. The role of mitochondrial respiration in the light has been the subject of considerable debate, but it is generally agreed that some TCA cycle activity is maintained to provide carbon skeletons for biosynthetic reactions (10, 24, 29). Use of radiolabeled TCA cycle intermediates has provided evidence for the maintenance of TCA cycle carbon flow in photosynthesizing cells (4, 20). Additional work has shown that during the assimilation of NH_4^+ and NO_3^- the flow of carbon through the TCA cycle increases to provide carbon skeletons for amino acid synthesis (9, 15, 18, 30).

Operation of the TCA cycle is dependent upon the oxidation of NADH and $FADH_2$ (17). This may occur via the mitochondrial electron transport chain, which results in O_2 consumption (22), or it may be coupled to biosynthetic reactions such as NO_3^- reduction (31). It is often assumed that during photosynthesis an increase in the ATP/ADP ratio would inhibit mitochondrial electron transport chain activity. Recent evidence, however, indicates that there is little difference in ATP/ADP ratios between light and dark (12, 27), and mass spectrometric analyses of O_2 exchange in green algae have shown that there is considerable activity of the mitochondrial electron transport chain during photosynthesis (23, 29).

In previous studies, we have demonstrated that the assimilation of NH_4^+ by photosynthesizing cells of *S. minutum* results in increased rates of both TCA cycle CO_2 release (3, 29) and mitochondrial O_2 consumption (29). This stimulation of TCA cycle carbon flow would result in an increase in the production of αKG, needed for NH_4^+ assimilation and net glutamate synthesis. The inhibition of NH_4^+-induced CO_2 release by cyanide and anaerobiosis was consistent with the mitochondrial electron transport chain oxidizing the NADH and $FADH_2$ produced during this increase in TCA cycle activity (29). In this study we present evidence which shows that NO_3^- and NO_2^- assimilation stimulate TCA cycle activity to a greater degree than does the assimilation of NH_4^+. We also show that a substantial portion of the reductant generated during this increase in TCA cycle activity appears to be exported from the mitochondrion to the chloroplast and used in support of NO_3^- and NO_2^- reduction both in the dark and during photosynthesis.

MATERIALS AND METHODS

Selenastrum minutum (Naeg.) Collins (Chlorophyta) (UTEX 2459) was grown in chemostat culture under N-limitation as previously described (7). Gross O_2 and CO_2 exchange were measured by mass spectrometry as described by Weger *et al.* (29), except that mixing employed a magnetic stirrer and $^{18}O_2$ was introduced as a bubble into the algal

[1] Supported by the Natural Sciences and Engineering Research Council of Canada. H. G. W. acknowledges a graduate fellowship from the School of Graduate Studies and Research of Queen's University.

[2] Abbrevations: TCA cycle, tricarboxylic acid cycle; DIC, dissolved inorganic carbon; GS, glutamine synthetase; GOGAT, glutamine 2-oxoglutarate aminotransferase; RuBP, ribulose 1,5-bisphosphate; Rubisco, ribulose 1,5-bisphosphate carboxylase oxygenase; αKG, αketoglutarate; OAA, oxaloacetate.

suspension. Dark O_2 consumption was measured using an O_2 electrode (Hansatech, King's Lynn, England).

Ammonium, nitrite, and nitrate assimilation were measured as N disappearance from the medium. Assimilation was measured using chemostat cells concentrated 2.5 times to a Chl concentration of approximately 5.3 $\mu g \cdot mL^{-1}$. Cells were placed in a water-jacketed (20°C) glass cuvette containing two serum stoppered sampling ports, one of which contained an O_2 electrode (YSI 5331, Yellow Springs, OH). Samples were withdrawn through the other port using 5 mL syringes. Samples were filtered through Whatman 934AH filters, and the filtrate was frozen until analysis. Ammonium was determined using an Orion 95-12 NH_3 electrode (Orion Instruments Inc., Cambridge, MA) for aerobic experiments. For anaerobic experiments the O_2 scavenging system (glucose/glucose oxidase/catalase) interfered with the operation of the NH_3 electrode; consequently, ammonium was determined colorimetrically according to Strickland and Parsons (27). Nitrate and nitrite were determined according to Strickland and Parsons (27), modified for flow through sample injection.

When required, anaerobiosis was achieved by adding glucose oxidase (final concentration 100 $\mu g \cdot mL^{-1}$) to a cell suspension containing 10 mM glucose and 20 $\mu g \cdot mL^{-1}$ catalase. Some anaerobic experiments in the light were conducted in the presence of DCMU to prevent O_2 production from water photolysis. DCMU was added to a final concentration of 20 μM, which was found to have no effect on O_2 consumption in either the light or dark. All inhibitors and glucose oxidase were obtained from Sigma Chemical Co. (St. Louis, MO).

Illumination for mass spectrometric and N assimilation experiments was provided at photon flux densities of 80 or 250 $\mu E \cdot m^{-2} \cdot s^{-1}$ (low and high light, respectively) by a tungsten light source. The higher photon flux density has been shown to be saturating for CO_2 fixation in this organism, while the lower rate is just subsaturating (7).

Steady state Chl *a* fluorescence was measured using a PAM fluorometer (Heinz Walz, Effeltrich FRG) as described by Turpin and Weger (28). Photochemical quenching ('Q quenching') was calculated according to Schreiber *et al.* (25). The effects of anaerobiosis on photochemical quenching were investigated by monitoring steady state fluorescence before and after adding glucose oxidase to an algal suspension (8 μg Chl $\cdot ml^{-1}$) containing glucose and catalase and illuminated at 110 $\mu E \cdot m^{-2} \cdot s^{-1}$. These experiments were undertaken both in the presence and absence of 2 mM $NaNO_3$.

Chl was measured by extraction in 100% methanol (7).

RESULTS

CO_2 Release

Addition of a nitrogen source (NH_4^+, NO_2^-, or NO_3^-) to N-limited cells of *S. minutum* resulted in a dramatic increase in the rate of TCA cycle CO_2 release (Table I). The assimilation of NO_3^- and NO_2^- resulted in higher rates of TCA cycle CO_2 release than did the assimilation of NH_4^+. In the dark the increase in TCA cycle CO_2 release during NO_3^- or NO_2^- assimilation is approximately 50% greater than that resulting from NH_4^+ assimilation. During photosynthesis the difference is even more pronounced, with both NO_3^- and NO_2^- producing at least twice the increase in CO_2 release caused by NH_4^+.

Table I. *Effects of N Assimilation on the Measured Rate of CO_2 Release*

N sources were added to a concentration of 1 mM, and the rate of $^{12}CO_2$ release into [^{13}C]DIC labeled medium was monitored for 15 min. All rates are the means of at least three experiments.

N Source	Dark	80 $\mu E \cdot m^{-2} \cdot s^{-1}$	250 $\mu E \cdot m^{-2} \cdot s^{-1}$
	$\mu mol\ CO_2\ released \cdot mg^{-1}\ Chl \cdot h^{-1}$		
Control	75	29	37
$+NH_4^+$	187	100	97
$+NO_2^-$	234	166	165
$+NO_3^-$	262	190	188

Table II. *Dark O_2 Consumption in Response to Different N Sources Added to a Concentration of 1 mM*

All rates are the means of at least three experiments.

N Source	O_2 Consumption Rate	Incremental Increase
	$\mu mol\ O_2 \cdot mg^{-1}\ Chl \cdot h^{-1}$	
Control	98	
$+NH_4^+$	223	125
$+NO_2^-$	132	34
$+NO_3^-$	113	15

O_2 Consumption

Dark O_2 consumption was stimulated by N assimilation, with NH_4^+ resulting in the largest stimulation, while NO_3^- and NO_2^- produced a relatively minor effect (Table II). These trends are also apparent during photosynthesis; NH_4^+ assimilation resulted in a large increase in the rate of gross O_2 consumption at both low and high light (Figs. 1A and 2A). NO_3^- and NO_2^- assimilation resulted in only a small stimulation of gross O_2 consumption at low light. At high light there is no effect of NO_3^- addition on O_2 consumption and the stimulation of O_2 consumption by NO_2^- was reduced (Fig. 1, B and C; Fig. 2, B and C).

Photosynthetic O_2 Evolution

The various N sources also differed in their effects on gross O_2 evolution. NH_4^+ assimilation resulted in a rapid and substantial decline in the rate of water photolysis, at both low and high light (Figs. 1A and 2A). In contrast, NO_3^- and NO_2^- assimilation did not affect the rate of gross O_2 evolution at low light (Fig. 1, B and C), and resulted in a slight stimulation of O_2 evolution at high light (Fig. 2, B and C). The combined changes in gross O_2 evolution and gross consumption during N assimilation resulted in net O_2 consumption in the light during NH_4^+ assimilation, but only minor changes in net O_2 exchange during assimilation of NO_3^- or NO_2^-. These trends in net O_2 exchange confirm those previously reported using an O_2 electrode (7).

N Assimilation

Culturing algae under N-limitation dramatically increases their capacity for N assimilation compared to N-sufficient

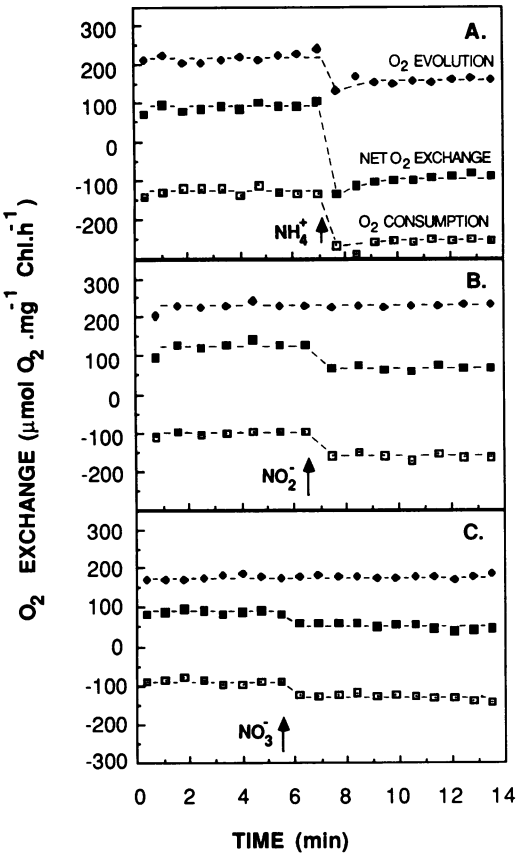

Figure 1. Effect of assimilation of different N sources on O_2 exchange at low light (80 $\mu E \cdot m^{-2} \cdot s^{-1}$). The arrow indicates addition of 1 mM N. A, NH_4^+; B, NO_2^-, C, NO_3^-. (⊡), Gross O_2 consumption; (♦), gross O_2 evolution; (■), net O_2 exchange.

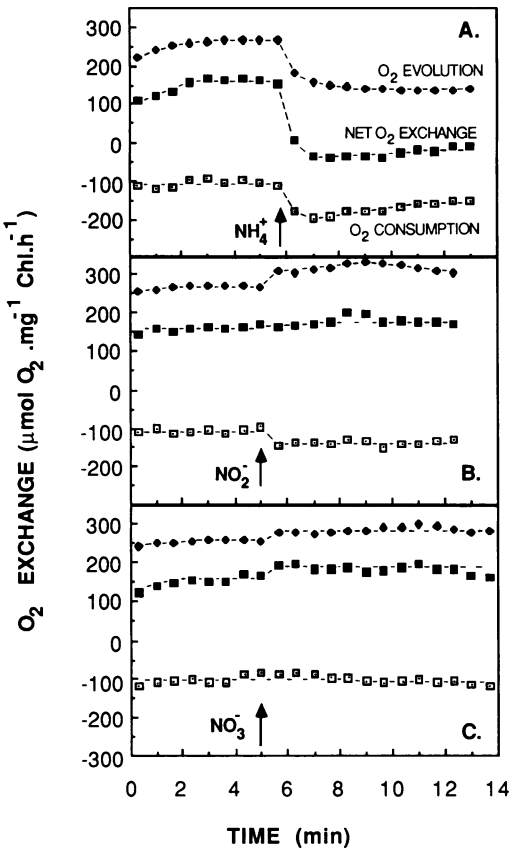

Figure 2. The effect of assimilation of different N sources on O_2 exchange at high light (250 $\mu E \cdot m^{-2} \cdot s^{-1}$). The arrow indicates addition of 1 mM N. A, NH_4^+; B, NO_2^-; C, NO_3^-. (⊡), Gross O_2 consumption; (♦), gross O_2 evolution; (■), net O_2 exchange.

cells (7). Under aerobic conditions, the rate of NH_4^+ assimilation by *S. minutum* is the same in the light and dark (approximately 170 μmol $NH_4^+ \cdot mg^{-1}$ Chl $\cdot h^{-1}$, Table III), and is unaffected by 20 μM DCMU. Anaerobiosis in either the light or dark caused a 70 to 80% decline in the rate of NH_4^+ assimilation. Under these conditions the presence of DCMU had only a slight inhibitory effect on NH_4^+ assimilation, indicating that the O_2 scavenging system was capable of maintaining functionally anaerobic conditions even during photosynthesis at low light (Table III).

The rates of NO_3^- and NO_2^- assimilation were greatest at high light (Table III). Addition of DCMU to aerobic cell suspensions at low light resulted in approximately a 55% decrease in the rate of NO_3^- assimilation relative to that in the absence of DCMU. The resulting rate was equal to that observed aerobically in the dark. Placing DCMU-treated illuminated cells under anaerobic conditions had no further inhibitory effect on the rate of NO_3^- assimilation. The presence of an O_2 scavenging system alone (in the absence of DCMU) had no effect on the rate of NO_3^- assimilation. In contrast, in the dark, anaerobiosis produced a 50% decline in NO_3^- assimilation relative to the aerobic rate (Table III). NO_2^- release to the medium was found during NO_3^- assimilation under all conditions, similar to reports for several other microalgae (2, 5). The average NO_2^- release rate under illuminated conditions was 34 μmol $NO_2^- \cdot mg^{-1}$ Chl $\cdot h^{-1}$, while

Table III. *Rates of N Assimilation under Various Conditions*

All rates are the means of at least two experiments.

Photon Flux Density	Treatment	N Source		
		NH_4^+	NO_2^-	NO_3^-
$\mu E \cdot m^{-2} \cdot s^{-1}$		*μmol N assimilated · mg^{-1} Chl · h^{-1}*		
250	Aerobic	170	160	155
80	Aerobic	167	117	127
80	Aerobic + DCMU	177	56	56
80	Anaerobic	49	81	133
80	Anaerobic + DCMU	31	43	57
Dark	Aerobic	169	55	53
Dark	Anaerobic	24	11	27

in the dark the rate increased to 63 μmol $NO_2^- \cdot mg^{-1} \cdot h^{-1}$. The rates of NO_3^- assimilation reported in Table III were calculated as NO_3^- disappearance minus NO_2^- release (*i.e.* net N assimilation).

The effects of these treatments on NO_2^- assimilation were similar to those observed with NO_3^-. The addition of DCMU to cells illuminated with low light resulted in a 50% decline in the NO_2^- assimilation rate, to a level equal to the dark aerobic rate (Table III). Placing illuminated, DCMU-treated cells under anaerobic conditions resulted in only a 20% decrease in the rate of NO_2^- assimilation compared to the aerobic rate. The same treatment, in the absence of DCMU,

produced comparable results. In the dark, however, anaerobiosis resulted in a 75% inhibition of NO_2^- assimilation (Table III).

Effects of Anaerobiosis on CO_2 Release during N Assimilation

Anaerobiosis reduced the control CO_2 release rate at low light to 6 μmol $CO_2 \cdot mg^{-1}$ Chl $\cdot h^{-1}$ (29) from control values of 29 μmol $CO_2 \cdot mg^{-1}$ Chl $\cdot h^{-1}$ (Table I). As shown in Table I, N assimilation resulted in a stimulation of TCA cycle CO_2 release. Subsequent anaerobiosis reduced the NH_4^+-stimulated rate to below aerobic control (Fig. 3A). In contrast, under anaerobic conditions, both NO_3^- and NO_2^- supported CO_2 release rates more than 4 times greater than aerobic control (Fig. 3A).

These experiments were repeated in the presence of DCMU to prevent water photolysis and thus ensure strict anaerobic conditions (Fig. 3B). The results are similar to those described above. NH_4^+-stimulated CO_2 release was reduced to below the aerobic control rate upon imposition of anaerobiosis, while both NO_3^- and NO_2^- assimilation supported much higher rates of anaerobic CO_2 release.

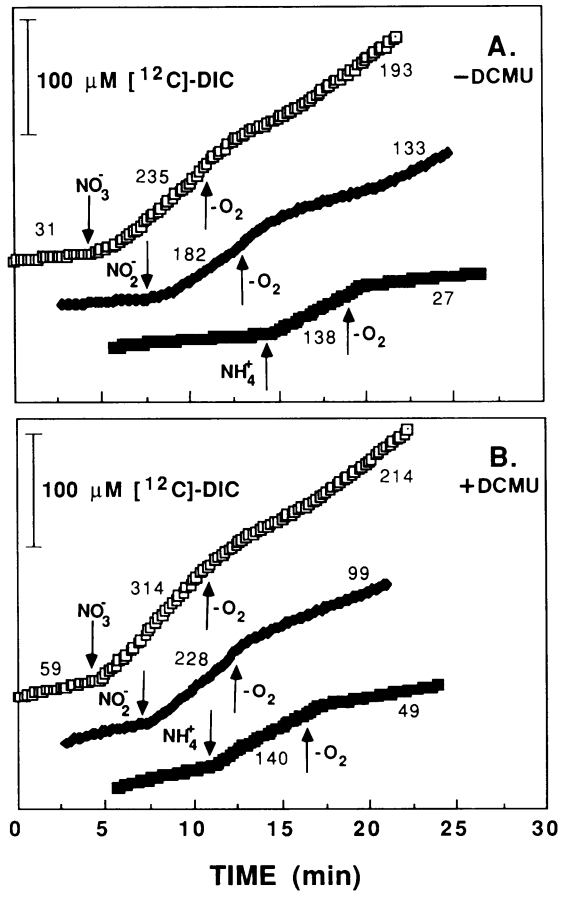

Figure 3. Effects of different N sources and anaerobiosis on respiratory $^{12}CO_2$ release during photosynthesis into medium containing 2 mM [^{13}C]-DIC. The first arrow indicates addition of 1 mM N, and the second arrow indicates the onset of anaerobiosis by addition of glucose oxidase; 10 mM glucose and 20 μg/mL catalase were added prior to the experiment. A, In the absence of DCMU; B, in the presence of DCMU.

Effect of Anaerobiosis on Photochemical Quenching

In control cells the onset of anaerobiosis produced a transient decline in photochemical quenching, which returned to aerobic control levels within 10 min. During NO_3^- assimilation, however, photochemical quenching declined from a mean aerobic level of 0.57 to 0.50 after 10 min of anaerobiosis. There was no recovery of photochemical quenching for the duration of the experiment. This indicates that the pool of reduced Q increases upon anaerobiosis during NO_3^- assimilation, suggesting a decrease in the requirements for photodriven electrons (16). This decline in photochemical quenching is not related to a decrease in the rate of O_2 photoreduction (Mehler reaction) caused by the addition of glucose oxidase, as DCMU or light/dark/light transitions had no effect on short term gross O_2 consumption (data not shown).

DISCUSSION

In both higher plants and green algae the assimilation of inorganic nitrogen proceeds predominately via the GS-GOGAT pathway (21). Net synthesis of glutamate by this pathway requires the operation of a portion of the TCA cycle to provide αKG for use in the GOGAT reaction. It is therefore not surprising that NH_4^+ addition to photosynthesizing cells results in an increase in TCA cycle carbon flow (15, 18, 30). Growth of the green alga *S. minutum* under N-limitation causes an increase in the capacity for N assimilation (3, 7). As a result, resupply of NH_4^+ produces a dramatic increase in TCA cycle carbon flow and CO_2 release in both the light and dark (Table I; Refs. 3, 29). This increase in TCA cycle activity implies that the rate at which NADH and $FADH_2$ are oxidized also increases. During NH_4^+ assimilation by N-limited *S. minutum*, this oxidation is coupled to the mitochondrial electron transport chain both in the dark and during photosynthesis (29).

As with NH_4^+ assimilation, NO_3^- and NO_2^- also increased the rate of TCA cycle carbon flow and CO_2 release (Table I; Refs. 3, 8). Surprisingly, the rate of CO_2 release observed during the assimilation of NO_3^- and NO_2^- was much greater than that observed with NH_4^+ (Table I). In fact, the ratio of CO_2 released/N assimilated was highest with NO_3^- and lowest with NH_4^+, implying greater TCA cycle activity during the assimilation of oxidized N sources (Table IV). This implied that the oxidation of TCA cycle reductant during NO_2^- and NO_3^- assimilation must occur at higher rates than during NH_4^+ assimilation.

One potential mechanism for this oxidation would be the mitochondrial electron transport chain and the coupled reduction of O_2 to H_2O. Unlike the case with NH_4^+, the assimilation of NO_3^- and NO_2^- resulted in only a small stimulation of O_2 consumption either in the dark (Table II) or during photosynthesis (Fig. 1, B and C; Fig. 2, B and C). This suggests that the mitochondrial electron transport chain is not as important in the oxidation of TCA cycle reductant during NO_3^- or NO_2^- assimilation as it is during the assimilation of NH_4^+. In fact, the ratio of CO_2 released/O_2 consumed varies greatly depending upon the N source being assimilated (Table IV). During NH_4^+ assimilation in the dark, the 'RQ' value was 0.84, a value within the theoretical range expected. When

Table IV. *Stimulation of CO_2 Release Relative to the Rates of N Assimilation (A) or, Relative to the Stimulation of O_2 Consumption Resulting from the Assimilation of Different N Sources (B)*

All ratios are expressed as μmol $CO_2 \cdot mg^{-1}$ Chl $\cdot h^{-1}/\mu$mol (N or $O_2) \cdot mg^{-1}$ Chl $\cdot h^{-1}$. The CO_2 released/O_2 consumed ratio (RQ) was determined using gross fluxes as calculated by mass spectrometry, and therefore differs from classical measures of RQ which employ net gas exchange measurements. The RQ value for NO_3^- at high light is undefined because no stimulation of gross O_2 consumption was observed (see Fig. 2C). Note that RQ values determined in the light are 'apparent RQ's,' biased by photosynthetic refixation of respired CO_2.

	Dark	80 $\mu E \cdot m^{-2} \cdot s^{-1}$	250 $\mu E \cdot m^{-2} \cdot s^{-1}$
A. CO_2 released/N assimilated (ratio: CO_2/N)			
NH_4^+	0.66	0.36	0.35
NO_2^-	2.89	1.06	0.80
NO_3^-	3.53	1.73	1.25
B. CO_2 released/O_2 consumed (RQ) (ratio: CO_2/O_2)			
Control	0.77	0.25	0.37
NH_4^+	0.84	0.43	0.76
NO_2^-	3.79	2.11	3.95
NO_3^-	8.13	4.17	Undefined

NO_2^- and NO_3^- were assimilated, however, the RQ increased to 3.79 and 8.13, respectively. The most logical explanation of these extremely high values is that the oxidation of TCA cycle reductant during the assimilation of NO_2^- and NO_3^- did not result in concomitant O_2 consumption. In other words, there must be an electron sink other than O_2, which is available during transient NO_3^- and NO_2^- assimilation, possibly NO_3^- and NO_2^- themselves. During photosynthesis, similar trends in the 'apparent RQ' were observed, once again suggesting the existence of alternative electron sinks.

During steady state photosynthesis and N assimilation, photogenerated reductant is thought to provide most or all of the reducing power for NO_3^- and NO_2^- reduction. However, examination of the rates of gross O_2 evolution suggest that during transient N assimilation by N-limited *S. minutum*, photogenerated reductant alone was insufficient to meet the demands of NO_3^- and NO_2^- reduction, which exceed those of carbon fixation. For example, during NO_3^- assimilation at high light the rate of gross O_2 evolution after NO_3^- addition (approximately 300 μmol $O_2 \cdot mg^{-1}$ Chl $\cdot h^{-1}$; Fig. 2C) indicated a rate of photogenerated reductant production of 600 μmol $(2e^-) \cdot mg^{-1}$ Chl $\cdot h^{-1}$. The simultaneous rate of NO_3^- reduction to NH_4^+ is 155 μmol $NO_3^- \cdot mg^{-1}$ Chl $\cdot h^{-1}$ (Table III), requiring 620 μmol $(2e^-) \cdot mg^{-1}$ Chl $\cdot h^{-1}$. The NO_2^- release rate was 34 μmol $NO_2^- \cdot mg^{-1}$ Chl $\cdot h^{-1}$, indicating additional reductant demands of 34 μmol $(2e^-) \cdot mg^{-1}$ Chl $\cdot h^{-1}$. An additional 155 μmol $(2e^-) \cdot mg^{-1}$ Chl $\cdot h^{-1}$ would be required for assimilation of NH_4^+ via GOGAT. The total reductant requirements (not including photosynthetic carbon assimilation) are therefore 809 μmol $(2e^-) \cdot mg^{-1}$ Chl $\cdot h^{-1}$, a value far in excess of that available from the light reactions of photosynthesis (600 μmol $(2e^-) \cdot mg^{-1}$ Chl $\cdot h^{-1}$). The occurrence of photosynthetic carbon fixation makes the reductant deficit even larger. We hypothesize that this deficiency in photosynthetic reducing power is overcome by TCA cycle reductant. During NH_4^+ assimilation, however, TCA cycle reductant is oxidized via the mitochondrial electron transport chain. A diagrammatic representation of this hypothesis is presented in Figure 4.

This hypothesis can be tested in several ways. If the mitochondrial electron transport chain is responsible for oxidizing TCA cycle reductant during NH_4^+ assimilation, but NO_3^- and NO_2^- fulfill this role during their assimilation (Fig. 4), then two predictions can be made. The first is that inhibition of the mitochondrial electron transport chain should result in a greater inhibition of TCA cycle CO_2 release during NH_4^+ assimilation than during the assimilation of NO_2^- or NO_3^-. Second, inhibition of the electron transport chain should result in a larger decrease in the rate of NH_4^+ assimilation than for either NO_3^- and NO_2^-.

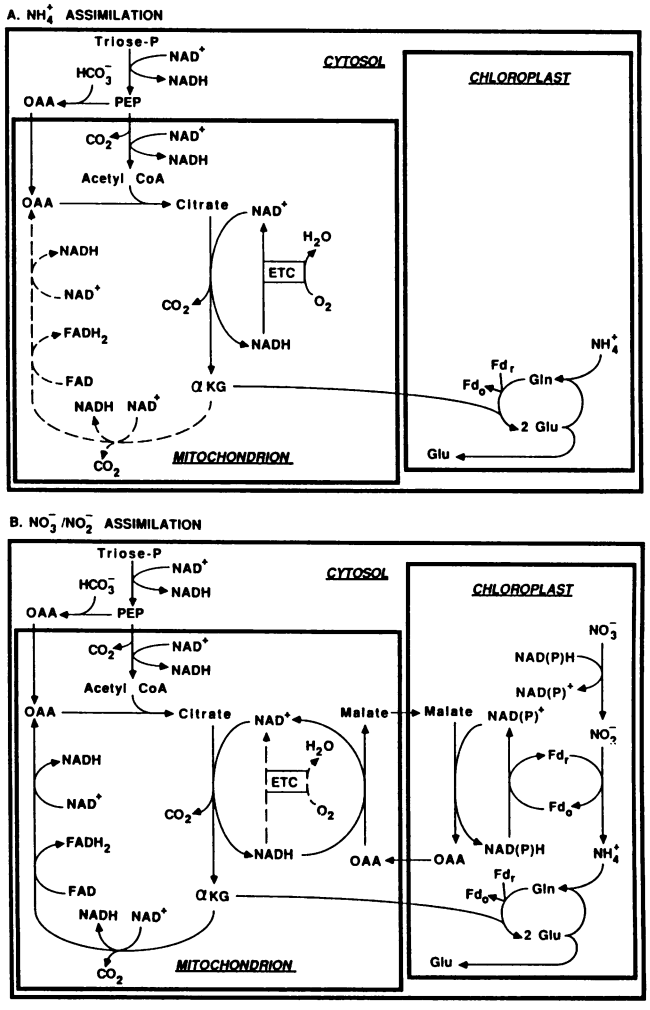

Figure 4. Proposed pathways of TCA cycle electron and carbon flow during transient NH_4^+ assimilation (A) and transient NO_3^-/NO_2^- assimilation (B) in the light by N-limited *S. minutum*. Cytosolic triose phosphate would be provided by the chloroplast, from the Calvin cycle and from starch breakdown in the light (8, 9). The actual mechanism of reductant shuttling during inorganic N assimilation is unknown, and has been represented in (B) as malate/oxaloacetate exchange across the mitochondrial and chloroplast envelopes. The light reactions of photosynthesis would also provide photogenerated reductant (NADPH and Fd_r), and therefore contribute to NO_3^-/NO_2^- reduction in the light (see "Discussion"). This is not illustrated. Abbreviations: αKG, αketoglutarate; OAA, oxaloacetate; Fd_r, reduced ferredoxin; Fd_o, oxidized ferredoxin.

Previous work with *S. minutum* has shown that inhibition of the mitochondrial electron transport chain via anaerobiosis inhibits both control and NH_4^+-stimulated CO_2 release from the TCA cycle (29). Anaerobiosis, however, allowed much greater rates of TCA cycle CO_2 release during NO_3^- and NO_2^- assimilation, both in the presence and absence of DCMU (Fig. 3, A and B). These results are consistent with oxidized N acting as an electron sink for TCA cycle reductant, thereby maintaining TCA cycle carbon flow and associated CO_2 release.

Since anaerobiosis results in a much lower rate of TCA cycle carbon flow during NH_4^+ assimilation than during the assimilation of oxidized N sources, this treatment should also substantially decrease NH_4^+ assimilation due to a lack of carbon skeletons for the GOGAT reaction. Consistent with this prediction, imposition of anaerobiosis (in the absence of DCMU) resulted in a much larger decrease in the rate of NH_4^+ assimilation than on the rates of NO_3^- or NO_2^- assimilation (Table III). The large decrease in the rate of NH_4^+ assimilation is probably a direct effect of the decrease in TCA cycle carbon flow, as ATP can still be produced via both cyclic and noncyclic photophosphorylation (Fig. 3A).

The imposition of strict anaerobiosis in the light by the combination of an O_2 scavenging system and 20 μM DCMU resulted in the additional complication of preventing the production of photogenerated reductant. The decrease in the rate of oxidized N assimilation in the light caused by DCMU (Table III) can be ascribed to this inhibition. Assimilation of oxidized N requires a large amount of reductant, and nitrite reductase is thought to be intimately associated with the photosynthetic electron transport chain which supplies reduced ferredoxin (11). The observation that the assimilation of oxidized N occurs in the absence of photogenerated reductant (in illuminated cells treated with DCMU, or in the dark) indicates that other reductant sources can provide electrons to both nitrate reductase and nitrite reductase. In spite of the complication caused by DCMU, anaerobiosis in the presence of DCMU resulted in an 82% decrease in the rate of NH_4^+ assimilation in the light, while NO_3^- assimilation was unaffected. NO_2^- assimilation was inhibited by only 20% (Table III).

Anaerobiosis in the dark greatly decreased the assimilation of all N sources (Table III). One possible explanation is the requirement for adequate ATP for N assimilation. In the dark, some mitochondrial electron transport chain activity is required for ATP generation, but in the light adequate ATP can be produced via cyclic photophosphorylation. The fact that NH_4^+ assimilation is dependent upon mitochondrial electron transport chain activity, but NO_3^- and NO_2^- assimilation are not, suggests that the decrease in NH_4^+ assimilation in the absence of mitochondrial electron transport chain activity during photosynthesis is not due to a limitation in ATP supply, but rather to a shortage of carbon skeletons for amino acid synthesis.

Interactions between the mitochondrion and the chloroplast during the assimilation of oxidized N should be detectable by monitoring changes in photochemical quenching of Chl *a* fluorescence. During NO_2^- assimilation the onset of anaerobiosis would remove one sink for TCA cycle reductant. Consequently, a diversion of mitochondrial reductant to NO_3^- and NO_2^- reduction in the chloroplast would be expected. Such a diversion should reduce the demand for photogenerated reductant, causing an increase in the pool of reduced Q and a decline in photochemical quenching. The data presented are consistent with this prediction.

An obvious characteristic of photosynthetic NH_4^+ assimilation is the large decrease in the rate of gross O_2 evolution (Figs. 1A and 2A). This occurs as a result of a decrease in RuBP concentration below the RuBP binding site density of Rubisco, causing RuBP limitation of photosynthetic carbon fixation (7, 9). The decreased rate of photosynthetic carbon fixation decreases the demand for photogenerated reductant and water photolysis. Suppression of carbon fixation also occurs during NO_3^- and NO_2^- assimilation (7, 9; HG Weger, DH Turpin, unpublished data), yet O_2 evolution is relatively unaffected (Figs. 1 and 2). Apparently this is a result of a diversion of photogenerated reductant from CO_2 fixation to the reduction of NO_3^- and NO_2^-, indicating that both photogenerated and TCA cycle reductant are utilized for the assimilation of oxidized N during photosynthesis. This is consistent with the partial inhibition of NO_3^- and NO_2^- reduction with DCMU.

In green algae both nitrite reductase and nitrate reductase are thought to be located in the chloroplast (11, 19). Since both the mitochondrial and chloroplastic inner membranes are impermeable to pyridine nucleotides, there must exist a shuttle mechanism that exports TCA cycle reductant from the mitochondrion to the chloroplast. Various dicarboxylate reductant shuttles have been proposed for both the mitochondrion (6, 14, 31) and the chloroplast (1, 13). However, in the current situation the shuttle would need to import reductant into the chloroplast during photosynthesis, the opposite of most of the proposed shuttle mechanisms. Such a system would allow the TCA cycle to operate both for the provision of the αKG needed for net glutamate synthesis and as a source of reducing power in addition to that provided by the light reactions of photosynthesis (Fig. 4). Even if nitrate reductase was located in the cytosol, as is thought for higher plants, reductant import by the chloroplast for NO_2^- reduction (the bulk of the electron demand) in the light would still be occurring. The actual shuttle mechanism is unknown, but may be based upon an OAA/malate system.

CONCLUSION

Evidence from this study suggests that in N-limited *S. minutum* TCA cycle reductant is exported from the mitochondrion to the chloroplast in support of transient NO_3^- and NO_2^- reduction both in the dark and during photosynthesis (Fig. 4). Assimilation of NH_4^+ results in an increase in the rate of TCA cycle CO_2 efflux in light and the dark, and a corresponding increase in mitochondrial O_2 consumption. NO_3^- and NO_2^- assimilation result in a much larger stimulation of TCA cycle CO_2 efflux than does NH_4^+ assimilation, but the effects on mitochondrial O_2 consumption are minor. We suggest that during NH_4^+ assimilation the TCA cycle operates primarily to provide carbon skeletons for amino acid biosynthesis, and the mitochondrial electron transport chain then serves to oxidize the TCA cycle reductant. During photosynthetic NO_3^- and NO_2^- assimilation, the TCA cycle operates both to provide carbon skeletons for amino acid bio-

synthesis and to provide reducing power for NO_3^- and NO_2^- reduction (Fig. 4). These results imply that in this organism mitochondrial respiration can supply reducing power to the chloroplast during photosynthesis under conditions where the production of photogenerated reductant is inadequate to meet metabolic demands.

ACKNOWLEDGMENTS

We wish to thank Drs. D. T. Canvin, D. T. Dennis, and R. D. Guy for useful discussions, and Drs. G. S. Espie, R. D. Guy, A. G. Miller, and Mr. D. G. Birch for valuable interactions in various aspects of mass spectrometry.

LITERATURE CITED

1. **Anderson JA, House CM** (1979) Polarographic study of oxaloacetate reduction by isolated pea chloroplasts. Plant Physiol **64:** 1058–1063
2. **Azuara MP, Aparicio PJ** (1984) Effects of light quality, CO_2 tensions and NO_3^- concentrations on the inorganic nitrogen metabolism of *Chlamydomonas reinhardii*. Photosynth Res **5:** 97–103
3. **Birch DG, Elrifi IR, Turpin DH** (1986) Nitrate and ammonium induced photosynthetic suppression in N-limited *Selenastrum minutum*. II. Effects of NO_3^- and NH_4^+ addition on CO_2 efflux in the light. Plant Physiol **82:** 708–712
4. **Chapman EA, Graham D** (1974) The effect of light on the tricarboxylic acid cycle in green leaves. II. Intermediary metabolism and the location of control points. Plant Physiol **53:** 886–892
5. **Collos Y** (1982) Transient situations in nitrate assimilation by marine diatoms. 2. Changes in nitrate and nitrite following a nitrate perturbation. Limnol Oceangr **27:** 528–535
6. **Ebbighausen H, Jia C, Heldt HW** (1985) Oxaloacetate translocator in plant mitochondria. Biochim Biophys Acta **810:** 184–199
7. **Elrifi IR, Turpin DH** (1986) Nitrate and ammonium induced photosynthetic suppression in N-limited *Selenastrum minutum*. Plant Physiol **81:** 273–279
8. **Elrifi IR, Turpin DH** (1987) The path of carbon flow during NO_3^--induced photosynthetic suppression in N-limited *Selenastrum minutum*. Plant Physiol **83:** 97–104
9. **Elrifi IR, Holmes JJ, Mayo WP, Weger HG, Turpin DH** (1988) RuBP limitation of photosynthetic carbon fixation: interaction between photosynthesis, respiration and ammonium assimilation in N-limited green algae. Plant Physiol **87:** 395–401
10. **Graham D** (1980) Effects of light on "dark" respiration. *In* DD Davies, ed, The Biochemistry of Plants, Vol 2. Academic Press, New York, pp 525–579
11. **Guerrero MG, Vega JM, Losada M** (1981) The assimilatory nitrate reducing system and its regulation. Annu Rev Plant Physiol **32:** 169–204
12. **Hampp R, Goller M, Füllgraf H, Eberle I** (1985) Pyridine and adenine nucleotide status, and pool sizes of a range of metabolites in chloroplasts, mitochondria and the cytosol/vacuole of *Avena* mesophyll chloroplasts during dark/light transitions: effect of pyridoxal phosphate. Plant Cell Physiol **26:** 99–108
13. **Hatch MD, Dröscher L, Flügge UI, Heldt HW** (1984) A specific translocator for oxaloacetate transport in chloroplasts. FEBS Lett **178:** 15–19
14. **Journet E-P, Neuberger M, Douce R** (1981) Role of glutamate oxaloacetate transaminase and malate dehydrogenase in the regeneration of NAD^+ for glycine oxidation by spinach leaf mitochondria. Plant Physiol **67:** 467–469
15. **Kanazawa T, Kirk MR, Bassham JA** (1970) Regulatory effects of ammonia on carbon metabolism in photosynthesizing *Chlorella pyrenoidosa*. Biochim Biophys Acta **205:** 401–408
16. **Krause GH, Weis E** (1984) Chlorophyll fluorescence as a tool in plant physiology. II. Interpretation of fluorescence signals. Photosynth Res **5:** 139–157
17. **Lambers H** (1985) Respiration in intact plants and tissues: it's regulation and dependence on environmental factors, metabolism and invased organisms. *In* R Douce, DA Day, eds, Encyclopedia of Plant Physiology, New Series Vol 18. Springer-Verlag, New York, pp 417–473
18. **Larsen PO, Cornwell KL, Gee SL, Bassham JA** (1981) Amino acid synthesis in photosynthesizing spinach cells. Effects of ammonia on pool sizes and rates of labelling from $^{14}CO_2$. Plant Physiol **68:** 292–299
19. **Lopez-Ruiz A, Verbelen JP, Roldan JM, Diez J** (1985) Nitrate reductase of green algae is located in the pyrenoid. Plant Physiol **79:** 1006–1010
20. **Marsh HV, Galmiche JM, Gibbs M** (1965) Effect of light on the tricarboxylic acid cycle in *Scenedesmus*. Plant Physiol **40:** 1013–1021
21. **Miflin BJ, Lea PJ** (1982) Ammonia assimilation and amino acid metabolism. *In* D Boulter, B Parthier, eds, Encyclopedia of Plant Physiology New Series, Vol 14A, Springer-Verlag, New York, pp 5–64
22. **Palmer JM, Møller IM** (1982) Regulation of NAD(P)H dehydrogenases in plant mitochondria. Trends Biochem Sci **7:** 258–261
23. **Peltier G, Thibault P** (1985) O_2 uptake in the light in *Chlamydomonas*. Evidence for persistent mitochondrial respiration. Plant Physiol **79:** 225–230
24. **Raven JA** (1984) Dark respiration. *In* Energetics and Transport in Aquatic Plants. Alan R. Liss, New York, pp 253–317
25. **Schreiber U, Schliwa U, Bilger W** (1986) Continuous reading of photochemical and non-photochemical chlorophyll fluorescence quenching with a new type of modulation fluorometer. Photosynth Res **10:** 51–62
26. **Stitt M, Lilley RN, Heldt HW** (1982) Adenine nucleotide levels in the cytosol, chloroplasts, and mitochondria of wheat leaf protoplasts. Plant Physiol **70:** 971–977
27. **Strickland JDH, Parsons TR** (1972) A Practical Handbook of Seawater Analysis, Ed 2. Fish Res Bd Can Bull 167, pp 71–89
28. **Turpin DH, Weger HG** (1988) Steady-state chlorophyll *a* fluorescence transients during ammonium assimilation by the N-limited green alga *Selenastrum minutum*. Plant Physiol **88:** 97–101
29. **Weger HG, Birch DG, Elrifi IR, Turpin DH** (1988) Ammonium assimilation requires mitochondrial respiration in the light: a study with the green alga *Selenastrum minutum*. Plant Physiol **86:** 688–692
30. **Woo KC, Canvin DT** (1980) Effect of ammonia on photosynthetic carbon fixation in isolated spinach leaf cells. Can J Bot **58:** 505–510
31. **Woo KC, Jokinen M, Canvin DT** (1980) Reduction of nitrate via a dicarboxylate shuttle in a reconstituted system of supernatant and mitochondria from spinach leaves. Plant Physiol **65:** 433–436