Dark Ammonium Assimilation Reduces the Plastoquinone Pool of Photosystem II in the Green Alga Selenastrum minutum

Narendranath Mohanty², Doug Bruce, and David H. Turpin*²

Department of Biology, Queen's University, Kingston, Ontario, K7L 3N6, Canada (N.M., D.H.T.);
Department of Biological Sciences, Brock University, St. Catharines, Ontario, L2S 3A1, Canada (D.B.)

ABSTRACT

The impact of dark NH₄⁺ and NO₃⁻ assimilation on photosynthetic light harvesting capability of the green alga Selenastrum minutum was monitored by chlorophyll a fluorescence analysis. When cells assimilated NH₄⁺, they exhibited a large decline in the variable fluorescence/maximum fluorescence ratio, the fluorescence yield of photosystem II relative to that of photosystem I at 77 kelvin, and O₂ evolution rate. NH₄⁺ assimilation therefore poised the cells in a less efficient state for photosystem II. The analysis of complementary area of fluorescence induction curve and the pattern of fluorescence decay upon microsecond saturating flash, indicators of redox state of plastoquinone (PQ) pool and dark reoxidation of primary quinone electron acceptor (Qₓ), respectively, revealed that the PQ pool became reduced during dark NH₄⁺ assimilation. NH₄⁺ assimilation also caused an increase in the NADPH/NADP⁺ ratio due to the NH₄⁺ induced increase in respiratory carbon oxidation. The change in cellular redoxant is suggested to be responsible for the reduction of the PQ pool and provide a mechanism by which the metabolic demands of NH₄⁺ assimilation may alter the efficiency of photosynthetic light harvesting. NO₃⁻ assimilation did not cause a reduction in PQ and did not affect the efficiency of light harvesting. These results illustrate the role of cellular metabolism in the modulating photosynthetic processes.

The assimilation of NH₄⁺ or NO₃⁻ by N-limited cells of Selenastrum minutum occurs at such high rates that photosynthetic carbon fixation does not meet the carbon demands for amino acid synthesis (14, 19). Under these conditions, photosynthetic carbon fixation is suppressed (3, 4), and the carbon demands are met by starch degradation (14, 19). During NH₄⁺ assimilation, the suppression of photosynthetic carbon fixation removes the sink for photosynthetic carbon and causes a decline in gross O₂ evolution (for a review, see ref. 21). Chl a fluorescence analysis, a sensitive indicator of photosynthetic processes, revealed that NH₄⁺ assimilation also results in a decline in the photochemical yield of PSII (8, 23). Recently, Turpin and Bruce (22) observed that NH₄⁺ assimilation by S. minutum caused a redistribution of excitation energy favoring PSI. These authors (22) suggested that this alteration aids in balancing the higher ATP/NAD(P)H requirement of NH₄⁺ assimilation relative to CO₂ fixation through PSI-supported cyclic photophosphorylation. NO₃⁻ assimilation, on the other hand, requires more NAD(P)H relative to ATP for its assimilation than NH₄⁺, and did not cause a suppression in O₂ evolution nor introduce any alteration in fluorescence characteristics of PSII or PSI (3, 4, 8, 22, 23).

NH₄⁺ assimilation in light influences the respiratory and photosynthetic carbon metabolism in an integrated fashion (18, 21, 24). In dark, N-limited S. minutum cells are capable of assimilating NH₄⁺ at a rate equivalent to that in light (21, 24). Dark NH₄⁺ assimilation influences carbon metabolism by stimulating glycolysis and mitochondrial respiration and enhancing dark anaerobic CO₂ fixation about 40-fold (24, 25). The impact of these metabolic alterations on subsequent photosynthetic behavior is not known. Understanding the interplay of photosynthetic, respiratory, and N metabolism requires an assessment on the effect of dark NH₄⁺ assimilation on photochemical functions. The present investigation reveals how NH₄⁺ assimilation in the dark poises S. minutum in a low efficiency photochemical state for PSII (state 2) and illustrates the regulatory effects of N assimilation and respiration on the light utilization processes of photosynthesis.

MATERIALS AND METHODS

Materials

The green alga Selenastrum minutum (Naeg) Collins (UEX2459) was cultured axenically in chemostats under NO₃⁻ limitation at a growth rate of 0.3 d⁻¹. Complete culture conditions were as described in Elrif and Turpin (3). The cells were harvested and resuspended at a density of 6 µg Chl mL⁻¹ in the supernatant and dark adapted for 30 min prior to experimental analysis.

Chl a Fluorescence Measurements

The room temperature Chl a fluorescence measurements were monitored at specified times during NH₄⁺ assimilation using a PAM fluorometer (Heinz Walz, Effeltrich, FRG). Fᵤ⁻3

* Abbreviations: Fᵤ, dark Chl fluorescence; Fᵥ, maximum fluorescence; Fᵥ, variable fluorescence, i.e., fluorescence rise from Fᵤ to Fᵥ; PQ, plastoquinone; Qₓ, primary quinone electron acceptor of PSI.

---

1 Supported by the Natural Sciences and Engineering Research Council grants to D.H.T. and D.B.
2 Current address: Department of Botany, University of British Columbia, Vancouver, B.C., V6T1W5.
was measured at 1.6 kHz. All other fluorescence measurements were done at 100 kHz.

Fluorescence induction kinetics and flash-induced fluorescence relaxation measurements were monitored by the PAM fluorometer as described by Schreiber (17). A saturating (1000 \( \mu \text{mol m}^{-2} \text{s}^{-1} \)) single turnover flash was fired from a XST 103 flash unit (Walz) while the PAM fluorometer time constant was kept at minimum value (zero position). The signal output was recorded on a digital storage oscilloscope (HM 208, HAMEG GmbH, Frankfurt, FRG) and printed on an x-y recorder (7035B, Hewlett-Packard). For these measurements, algal cells were resuspended in supernatant (algal media) after cell harvesting. The temperature was kept at 20°C during fluorescence analysis.

The 77 K fluorescence emission spectral analyses were done with a laboratory assembled fluorometer based on an optical multichannel analyzer (EG&G PARC, Princeton, NJ) as described previously (22). At specific times during NH₄⁺ assimilation in dark, 100 \( \mu \text{L} \) samples were removed into capillary tube sample holders and frozen in liquid N₂, and the emission spectra recorded. The relative fluorescence yield of PSI to PSII was expressed as the ratio of fluorescence yield at 686 nm to that at 717 nm.

Other Measurements

Net \( \text{O}_2 \) exchange was monitored with a Clarke type \( \text{O}_2 \) electrode (Hansatech Ltd., Kings Lynn, England). The photosynthetic electron transfer activity was measured as net \( \text{O}_2 \) exchange at an irradiance of about 220 \( \mu \text{mol m}^{-2} \text{s}^{-1} \) and the respiratory activity was measured as \( \text{O}_2 \) consumption in dark.

NH₄⁺ assimilation was assayed as the disappearance of NH₄⁺ from the assay medium at specific time intervals after addition of 1 mM NH₄⁺. The NH₄⁺ content was measured using the phenol nitroprusside hypochlorite method of Strickland and Parsons (20).

The cellular NADP/H was analyzed spectrophotometrically using an enzymatic cycling assay as described by Passonneau and Lowry (15).

The total Chl (a + b) was estimated spectrophotometrically following extraction in methanol (7).

RESULTS

In \( N \)-limited cells of \( S. \ minutum \), \( F_o \) was obtained with the onset of the weak modulated measuring beam, and \( F_m \) was attained with the application of a 1 sec saturating flash of about 1100 \( \mu \text{mol m}^{-2} \text{s}^{-1} \) intensity. \( F_o \) and \( F_m \) were monitored at specified times following 1 mM NH₄⁺ addition (Fig. 1A). \( F_m \) and more specifically the \( F \) yield was reduced by 40% of control value, when measured at 6 and 15 min after the onset of NH₄⁺ assimilation. Thereafter, \( F \) recovered gradually and was almost restored after 60 min of NH₄⁺ addition in dark (Fig. 1A). This recovery corresponded with the completion of NH₄⁺ assimilation. The presence of azaserine, an inhibitor of NH₄⁺ assimilation, arrested the NH₄⁺-induced alteration of fluorescence (Fig. 1B).

Figure 2 correlates the pattern of NH₄⁺ assimilation with Chl a fluorescence represented as the \( F/F_0 \) and \( F_{686}/F_{717} \) ratios, photosynthetic \( \text{O}_2 \) evolution, and respiratory \( \text{O}_2 \) uptake activity measured in samples taken from the experimental cuvette at specific time intervals after addition of NH₄⁺ in dark. The \( F/F_0 \) ratio, an indicator of photochemical yield of PSII, dropped from a control value of 0.65 to 0.47 during the first 15 min after NH₄⁺ resupply and slowly recovered corresponding to the completion of NH₄⁺ assimilation (Fig. 2A.
and B). The 77 K Chl a fluorescence yield of PSII relative to
that of PSI (F_{680}/F_{717}) dropped markedly and recovered once
the added NH_4^+ was assimilated (Fig. 2B). Net O_2 evolution
by briefly illuminated dark NH_4^+ assimilating cells exhibited
a similar pattern of initial decline and subsequent gradual
restoration (Fig. 2C). The dark respiratory O_2 uptake showed
more than a twofold increase during NH_4^+ assimilation in
dark with a return to control value once the NH_4^+ assimilation
was complete (Fig. 2C).

The Chl a fluorescence induction kinetics of N-limited
control cells and cells assimilating NH_4^+ in dark are shown
in Figure 3. The area bound by the fluorescence rise and its
asymptote (complementary area) is a measure of the pool size
of electron acceptors of PSII, mainly the pool of PQ (1). The
PQ pool size is larger than the pool size of photochemical
centers of PSII and remains in a predominant oxidized state
in dark-adapted photosynthetic systems. NH_4^+ assimilating
cells exhibited a much faster fluorescence rise than control
cells, thus a smaller complementary area (Fig. 3A), indicating
that the PQ pool in NH_4^+ assimilating cells was in a reduced
state prior to illumination. In contrast, assimilation of 1 mM
NO_3^- did not alter the fluorescence induction rise pattern
(Fig. 3B). The fluorescence induction kinetics of dark NH_4^+
assembling cells analyzed at specified times revealed that the
alteration in complementary areas of fluorescence curve (i.e.
change in PQ redox state) is reversible. Once NH_4^+ is assimil-
ated, the cells gradually regained the normal fluorescence rise
kinetics (Fig. 4), indicating the return of the PQ pool to its
typical oxidized dark state.

Analysis of rapid fluorescence relaxation kinetics following
a saturating single turnover flash allows measurement of the
reoxidation kinetics of Q_x (17). In dark-adapted control cells,
a single saturating flash raises the fluorescence to F_{m}',
indicating the reduction of Q_x (Fig. 5A). Following the flash,
the fluorescence relaxes, revealing the complex decay kinetics
of Q_x reoxidation (Fig. 5A). The addition of 20 μm DCMU, a

---

**Figure 2.** A, Dark NH_4^+ assimilation as a function of time; B, concur-
rent suppression of Chl a fluorescence yield; and C, net O_2 exchange
in light (C) and dark respiratory O_2 uptake (■) activity in μmol·mg^-1
Chl·h^-1 in N-limited cells of S. minutum.

**Figure 3.** Effect of nitrogen assimilation on the Chl a fluorescence
induction kinetics of N-limited cells of S. minutum. The fluorescence
rise kinetics are of cells assimilating (A) NH_4^+ or (B) NO_3^- for 5 min in
dark. Traces are matched with F_m for those of control cells.

**Figure 4.** The pattern of Chl a fluorescence induction kinetics of (A)
N-limited cells of S. minutum and (B–F) cells assimilating NH_4^+ in dark
monitored at specified times after addition of 1 mM NH_4^+. The time
course of NH_4^+ assimilation was identical to that shown in Figure
2the digits shown are the complementary area of the fluorescence
induction curve (hatched for A and B only) expressed as a percentage
of control value.
known inhibitor of electron transfer from QA, completely abolished this dark relaxation of fluorescence (Fig. 5B). Thus, this rapid fluorescence decay assay, if applied to test the effect of dark NH₄⁺ assimilation on the redox state of PSII acceptor components. In cells assimilating NH₄⁺ in dark, the fast fluorescence relaxation following a flash was largely arrested (Fig. 5C). N-limited cells assimilating NO₃⁻ in dark, however, showed normal dark fluorescence relaxation properties (Fig. 5D). The capacity of PQ to reoxidize QA, represented as the magnitude of fluorescence relaxation occurring with 3 ms of flashing, was monitored during the period of NH₄⁺ assimilation, and is presented in Figure 6. The NH₄⁺ assimilating cells showed only 20 to 30% of QA reoxidation ability compared to 75% observed in control cells. This arrest of dark QA reoxidation by PQ recovered following the completion of NH₄⁺ assimilation (Figs. 2 and 6).

**DISCUSSION**

Chl a fluorescence and O₂ exchange analyses have revealed that dark NH₄⁺ assimilation by N-limited cells of *S. minutum* results in a lower PSII photochemical yield relative to control cells (Figs. 1 and 2). This NH₄⁺-induced loss of PSII activity recovered upon the completion of NH₄⁺ assimilation. The lack of NH₄⁺ effect in the presence of azaserine, an inhibitor of NH₄⁺ assimilation (Fig. 1), shows that the positioning of cells in a low photoactive PSII state is the result of NH₄⁺ assimilation and not of the presence of NH₄⁺ per se. Our Chl a fluorescence analysis revealed that NH₄⁺ assimilation influences the dark step of electron transfer path between PSII and PSI and showed that NH₄⁺ assimilating cells, unlike control cells, lack the ability to reoxidize QA in dark (Figs. 3-6). This observation suggests that the dark NH₄⁺ assimilation causes reduction of the PQ pool. These changes are completely reversible, recovering after NH₄⁺ assimilation is complete (Figs. 1-6).

High rates of dark NH₄⁺ assimilation into amino acids is sustained by the mobilization of starch to provide carbon skeleton and reducing power (14, 19, 24). The onset of NH₄⁺ assimilation changes the ADP/ATP ratio and the concentration of glutamate and glutamine which in turn activate glycolytic carbon flow (13, 18, 24, 25). The increase in both glycolytic and oxidative pentose phosphate pathway activity will increase cellular NAD(P)H production. Preliminary analysis of cellular NADPH revealed 16% increase in NADPH/NADP⁺ for the cells assimilating NH₄⁺ for 10 min in dark (data not shown). This condition is reported to cause the reduction of components of the intersystem photosynthetic electron transfer chain, driving the cells to a low photoactive state for PSII, or state 2 (1, 2, 5, 10, 11). Recently, it has been reported that a thylakoid bound NAD(P)H dependent PQ reductase activity was capable of reducing PQ in dark and constitutes a chloroplast respiratory path for carbon oxidation (1, 2, 5, 6, 16, 26). This may provide a mechanism by which elevated levels of NADPH, brought about by increased respiratory carbon metabolism, may serve to reduce the PQ pool.

NO₃⁻ reduction and assimilation has a higher demand for NADPH than does NH₄⁺. It is therefore not surprising that NO₃⁻ assimilation caused a decline in NADPH levels (data not shown). As would be expected, dark NO₃⁻ assimilation therefore did not cause PQ reduction or a decline in QA reoxidation and PSII photoactivity, (Figs. 3 and 5). This is consistent with our earlier observations (3, 4, 8, 22, 23).

We have demonstrated that reduction of PQ during dark NH₄⁺ assimilation controls the state transition mechanism. The state transition is most often thought of as a regulatory mechanism which serves to optimize the relative activities of PSII and PSI under conditions of adverse environmental light quality. In this work we have shown that the mechanism is light independent *in vivo*. We have demonstrated a reversible transition to state 2 triggered by reduction of PQ in the dark driven by NH₄⁺ assimilation. These results, along with the recent observation that cellular metabolism can influence the PSII yield (2, 5, 8, 9, 11, 12, 22), further strengthen the evidence for metabolic feedback playing an important role in the regulation of the light utilizing steps of photosynthesis.

![Figure 5](image_url)

**Figure 5.** Analysis of rapid Chl a fluorescence decay kinetics following a saturating single turnover flash (half-peak width 3 μs) in control N-limited cells of *S. minutum* (A), cells with 20 μM DCMU (B), and cells assimilating NH₄⁺ (C) or NO₃⁻ (D), for 5 min in dark.

![Figure 6](image_url)

**Figure 6.** The capacity of PQ pool to reoxidize QA within 3 ms following a saturating single turnover flash, monitored after N-limited cells of *S. minutum* were resupplied with 1 mM NH₄⁺ in dark. Control cells (i.e. before NH₄⁺ addition) are marked as 0 min samples.
ACKNOWLEDGMENTS

We wish to thank our colleagues Drs. Greg C. Vanlerberghe for the NADP/H measurements and Ronald G. Smith for stimulating discussion; and Jo-Anne Lorway for preparation of the manuscript.

LITERATURE CITED