Plant Physiol. (1988) 88, 97–101
0032-0889/88/88/0097/05/$01.00/0

Steady-State Chlorophyll *a* Fluorescence Transients during Ammonium Assimilation by the N-Limited Green Alga *Selenastrum minutum*[1]

Received for publication January 1, 1988 and in revised form April 4, 1988

DAVID H. TURPIN* AND HAROLD G. WEGER
Department of Biology, Queen's University, Kingston, Ontario, Canada K7L 3N6

ABSTRACT

The assimilation of ammonium by the N-limited green alga *Selenastrum minutum* results in the suppression of photosynthetic electron flow from H_2O to CO_2 (6, 7, 18). In this study, results are presented which describe the correponding change in steady-state chlorophyll *a* fluorescence. The addition of ammonium resulted in a transient decline in fluorescence followed by a marked increase. Fluorescence did not return to control levels until the added ammonium had been assimilated. Analysis of the fluorescence transients showed that ammonium assimilation resulted in a rapid increase in nonphotochemical quenching (Q_e) peaking 10 to 15 seconds after ammonium addition. Q_e then decreased dramatically reaching a minimum value approximately 45 seconds following ammonium addition and returned to the control level only after the added ammonium had been assimilated. There were no effects of ammonium addition on photochemical quenching (Q_q) for approximately 10 to 15 seconds at which time both gross O_2 evolution (as measured by mass spectrometry) and Q_q declined. In the presence of D,L-glyceraldehyde or when cells were held at the CO_2 compensation point, the addition of ammonium resulted in a decline in Qe 10 to 15 seconds after addition. The Q_e peak and the Q_q decline were absent. These results imply that the transient increase in Q_e and the subsequent decline in Q_q may be attributed to the decline in Calvin cycle activity during ammonium assimilation. The decline in Q_e is apparently a direct result of ammonium assimilation. The observation that the Q_e peak precedes the Q_q decline would be consistent with the decreases in Calvin cycle carbon flow occurring at the kinase reactions prior to glyceraldehyde-3-phosphate dehydrogenase.

The assimilation of ammonium by photosynthetic tissues results in the redirection of recent photosynthate from the synthesis of starch and sucrose to the synthesis of amino acids (2, 6, 7, 9, 11, 16, 21). When algae are grown under N-limitation, the maximum potential rate of ammonium assimilation often increases (6, 18, 20). It is therefore not surprising that the effects of ammonium assimilation on photosynthetic and respiratory metabolism increase dramatically when the cells are grown under N-limiting conditions (6, 12, 17).

In studies with the N-limited green alga *Selenastrum minutum*, we have demonstrated that the assimilation of ammonium during photosynthesis results in a large increase in the rate of TCA[2] cycle CO_2 release and mitochondrial O_2 consumption (3, 18). These gas exchange characteristics are a result of the increase in carbon flow through the TCA cycle in support of amino acid biosynthesis and the corresponding increase in mitochondrial electron transport. Accompanying these changes in respiratory metabolism is a decrease in photosynthetic carbon fixation and gross O_2 evolution (6, 18). This decrease in photosynthetic carbon fixation is due in part to a decrease in the RuBP concentration below the RuBP binding site density of ribulose bisphosphate carboxylase/oxygenase (8). Therefore, during the assimilation of ammonium by N-limited *S. minutum*, photosynthetic carbon fixation is limited by RuBP. We have demonstrated that these changes in metabolism are dependent upon the assimilation of ammonium into amino acids and are not due to uncoupling (3, 6–8, 16, 18) but at present the regulatory mechanisms controlling these changes are unknown and further work is required before a comprehensive model is established.

Recent advances in the measurement of steady-state Chl fluorescence (13) enable examination of the state of photosynthetic electron flow from H_2O to CO_2 (14, 19). Given the marked changes in photosynthesis during ammonium assimilation, the accompanying changes in steady-state fluorescence may provide further insights into this phenomenon. This study reports changes in steady-state fluorescence, Q_q and Q_e, and photosynthetic O_2 evolution and CO_2 fixation that occur during the assimilation of ammonium by N-limited cells of *S. minutum*. These results have implications for the mechanism by which N-assimilation alters photosynthetic carbon metabolism.

MATERIALS AND METHODS

Organism and Culture. *Selenastrum minutum* was isolated from Lake Ontario, Kingston, Canada in 1982. This strain is now maintained in the University of Texas Culture Collection (UTEX 2459). It was cultured in NO_3^-limited chemostats as previously described (6).

Measurement of Steady-State Fluorescence. For all experiments, with the exception of those involving MS, algae (7–8 μg Chl $a \cdot mL^{-1}$) were contained in a Hansatech O_2 electrode chamber (Kings Lynn, England), and ammonium chloride was added to a concentration of 100 μM. Steady-state fluorescence was measured using a PAM fluorometer (Heinz Walz, FRG). The operation of this system has been described in considerable detail elsewhere (13). In brief, a low intensity measuring beam pulses

[1] Supported by the Natural Sciences and Engineering Research Council of Canada.

[2] Abbreviations: TCA, tricarboxylic acid; RuBP, ribulose bisphosphate; Q_q, photochemical quenching; Q_e, nonphotochemical quenching; F_v, variable fluorescence; $(F_v)_m$ maximum variable fluorescence $(F_v)_s$, flash-saturated variable fluorescence.

at 100 kHz. The resulting fluorescence is detected by a photodiode and only the 100 kHz fluorescence is amplified, yielding a measure of variable fluorescence (F_v). Thus, measured changes in fluorescence reflect changes in photochemistry and are not due to changes in the actinic light intensity. Using the same principle, F_o may be measured prior to turning on the actinic light. The maximum variable fluorescence $(F_v)_m$ is determined by turning on a saturating actinic light and recording the maximum fluorescence yield during the Kautsky transient. In our system, this method for determining $(F_v)_m$ agreed within 10% of the value determined in the presence of DCMU. Once the monitoring of steady-state fluorescence was initiated, saturating flashes of 1 s duration were applied every 10 s. This served to fully reduce Q and provided an estimate of the flash-saturated variable fluorescence $(F_v)_s$ (13). Figure 1B provides a graphical representation of the fluorescence response of this system and the determination of F_o, F_v, and $(F_v)_s$.

Calculation of Fluorescence Quenching. The continual monitoring of F_v and $(F_v)_s$ allowed for the determination of continuous plots of Q_q and Q_e. Q_q and Q_e were calculated using the equations of Schreiber *et al.* (13), where:

$$Q_e = \frac{(F_v)_m - (F_v)_s}{(F_v)_m}$$

$$Q_q = \frac{(F_v)_s - F_v}{(F_v)_s}.$$

MS. Measurement of gross photosynthetic O_2 evolution was determined as previously described (18). To facilitate the simultaneous measurement of fluorescence and O_2 evolution, cell densities were increased to 18 μg Chl $a \cdot mL^{-1}$. Ammonium chloride was added to a concentration of 200 μM. The fiberoptic probe of the PAM fluorometer was connected directly to the mass spectrometer sample cuvette.

Ammonium Analysis. Culture samples (0.3 mL) were removed from the cuvette and immediately frozen in liquid N_2. Samples were then thawed and frozen two times to permeabilize the cells. This ensured that the measured decrease in ammonium could be attributed to assimilation and not just to uptake. Ammonium was analyzed as described by Strickland and Parsons (15).

Other Procedures. Measurements of photosynthetic carbon fixation and the use of the glutamine:oxoglutarate aminotransferase inhibitor azaserine were undertaken as described by Elrifi and Turpin (6). Compensation point experiments were undertaken as described by Birch *et al.* (3). D,L-Glyceraldehyde was added to a concentration of 25 mM and cells were preincubated for 30 min in the dark.

RESULTS

Effects of NH_4^+ Assimilation on F_v, O_2 Evolution, and Carbon Fixation. The addition of ammonium (200 μM) to steady-state photosynthesizing cells of *S. minutum* (photon flux density of 690 $\mu E \cdot m^{-2} \cdot s^{-1}$) resulted in major changes in the steady-state F_v (Fig. 1B). Immediately following ammonium addition there was a drop in F_v that lasted approximately 10 to 15 s. This was followed by a rapid increase in F_v, which reached a peak approximately 45 s after ammonium addition. Fluorescence then slowly decayed back to the control level. Although F_v returned to near control values following the assimilation of ammonium, minor oscillations continued for several minutes. Simultaneous measurement of $^{16}O_2$ evolution from water photolysis showed that ammonium addition resulted in transient suppression in photodriven electron flow from H_2O to CO_2. Parallel experiments showed that photosynthetic carbon fixation decreased from a control value of 220 μmol $C \cdot mg^{-1}$ Chl $a \cdot h^{-1}$ to 130 μmol $C \cdot mg^{-1}$ Chl $a \cdot h^{-1}$ following ammonium addition. This is consistent with the previously reported effects of ammonium assimilation on photosynthetic O_2 evolution (18) and photosynthetic carbon fixation in this organism (6, 18).

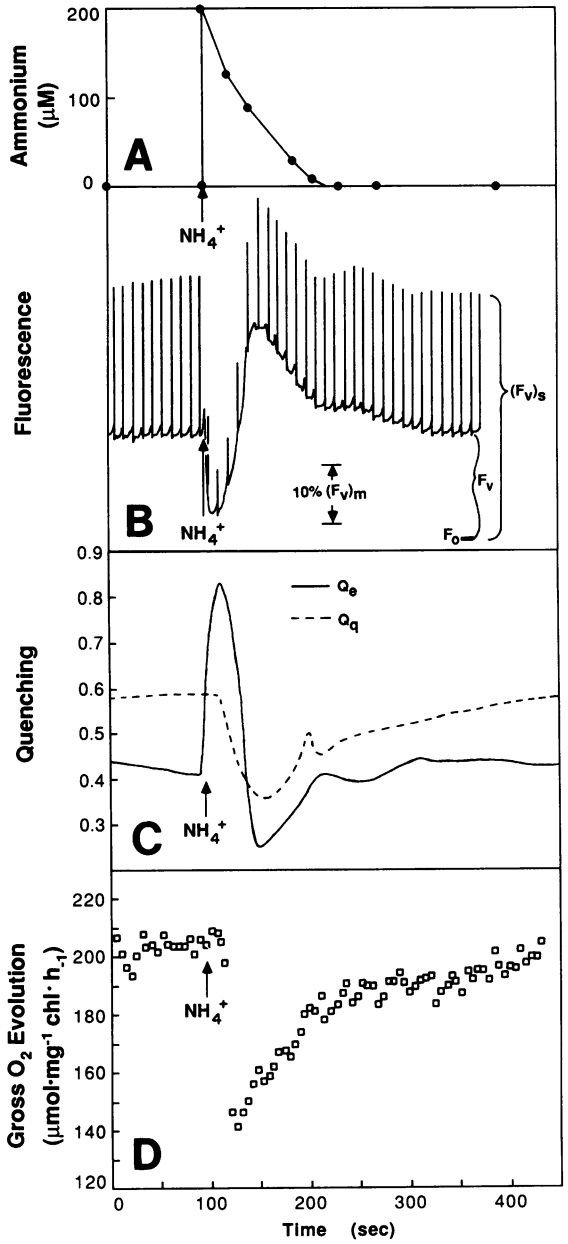

FIG. 1. Effects of ammonium addition (200 μM) to N-limited *S. minutum* on steady-state fluorescence, fluorescence quenching and gross photosynthetic oxygen evolution. (A) The time course of ammonium assimilation; (B) resulting changes in steady-state fluorescence (F_v) and flash-saturated fluorescence $\{(F_v)_s\}$ (the position of F_o is as indicated); (C) Q_e and Q_q derived from the data in (B) using the equations of Schreiber *et al.* (13); (D) simultaneous changes in gross photosynthetic oxygen evolution measured by MS.

Changes in Fluorescence Quenching during NH_3 Assimilation. Analysis of the changes in steady-state fluorescence (F_v) and the flash-saturated fluorescence $\{(F_v)s\}$ allow for the calculation of both Q_q and Q_e. Ammonium addition resulted in a rapid increase in Q_e, which peaked after approximately 10 to 15 s (Fig. 1C). Q_e then collapsed, reaching its lowest point approximately 45 s after ammonium addition (Fig. 1C). Q_e then recovered to its original value once the added ammonium had been assimilated. There were no effects on Q_q until approximately 10 to 15 s following

ammonium addition (Fig. 1C). At this time, Q_q decreased and remained low until the added ammonium was assimilated (Fig. 1C). The decrease in Q_q coincided with the decrease in photosynthetic O_2 evolution.

The addition of ammonium therefore resulted in three distinct changes in fluorescence quenching. The first was a rapid increase in Q_e, the second a decrease in Q_q, which was accompanied by the third, a decrease in Q_e. We have termed these three transients the Q_e peak, the Q_q decline, and the Q_e decline, respectively.

Role of NH_4^+ Assimilation. Azaserine is an inhibitor of the ammonium assimilating enzyme glutamine:oxoglutarate aminotransferase. Pretreatment of *S. minutum* with this inhibitor results in the inhibition of both ammonium assimilation and the net synthesis of amino acids (18). The addition of ammonium to azaserine-treated cells resulted in no change in the fluorescence characteristics of the culture (data not shown).

Effects of Irradiance. *Q_e Quenching.* Increases in photon-flux density, from a limiting value (88 $\mu E \cdot m^{-1} \cdot s^{-1}$) to a supersaturating value (870 $\mu E \cdot m^{-2} \cdot s^{-1}$), resulted in increases in the steady-state level of Q_e (Fig. 2). The addition of ammonium caused both a Q_e peak and subsequent decline at each irradiance (Fig. 2).

Q_q Quenching. At the two lowest irradiances, Q_q declined during ammonium assimilation. At supersaturating light, the steady-state level of Q_q was reduced significantly and declined only slightly in response to ammonium addition (Fig. 2C).

The apparent differences in the quenching curves reported in Figures 1 and 2 are due to the greater cell densities used in the mass spectrometer experiments and the different geometry of the sample cuvettes.

Effect of NH_4^+ Concentration. A doubling of the added ammonium concentration resulted in similar changes in fluorescence with the exception that the fluorescence peak was of longer duration (data not shown). This is due to the greater time required for the cells to assimilate the higher concentration of ammonium.

Relationship between Changes in Photosynthesis and Fluorescence. The ammonium-induced changes in photosynthetic carbon fixation and O_2 evolution must be responsible for a major component of the observed changes in fluorescence. The magnitude of this contribution was evaluated by examining the ammonium-induced changes in quenching under conditions where carbon fixation was relatively unaffected by ammonium assimilation. This was accomplished in two ways. The first involved inhibiting the Calvin cycle with D,L-glyceraldehyde while the second involved washing the cells with dissolved inorganic carbon-free buffer and allowing them to reach their CO_2 compensation point.

The control rate of carbon fixation (220 μmol $CO_2 \cdot mg^{-1}$ Chl $\cdot h^{-1}$) declined by 90 μmol $CO_2 \cdot mg^{-1}$ Chl $\cdot h^{-1}$ during ammonium assimilation. In the presence of D,L-glyceraldehyde, the control rate of photosynthesis was reduced to 72 μmol $CO_2 \cdot mg^{-1}$ Chl $\cdot h^{-1}$. The addition of ammonium resulted in only a 15 μmol $\cdot mg^{-1}$ Chl $\cdot h^{-1}$ decline in this rate. During the assimilation of ammonium by these cells, there was little change in fluorescence quenching for the first 10 to 15 s, after which time Q_e quenching declined dramatically (Fig. 3). Both the Q_e peak and Q_q decline were absent.

When *S. minutum* is held at the CO_2 compensation point, ammonium assimilation proceeds, but there is no decrease in photosynthetic carbon fixation upon ammonium addition (3). Under such conditions, there was no effect of ammonium addition on fluorescence quenching for the first 10 to 15 s, following which Q_e declined (Fig. 4). Ammonium addition to these cells did not cause a Q_e peak, and its effects on Q_q were relatively minor.

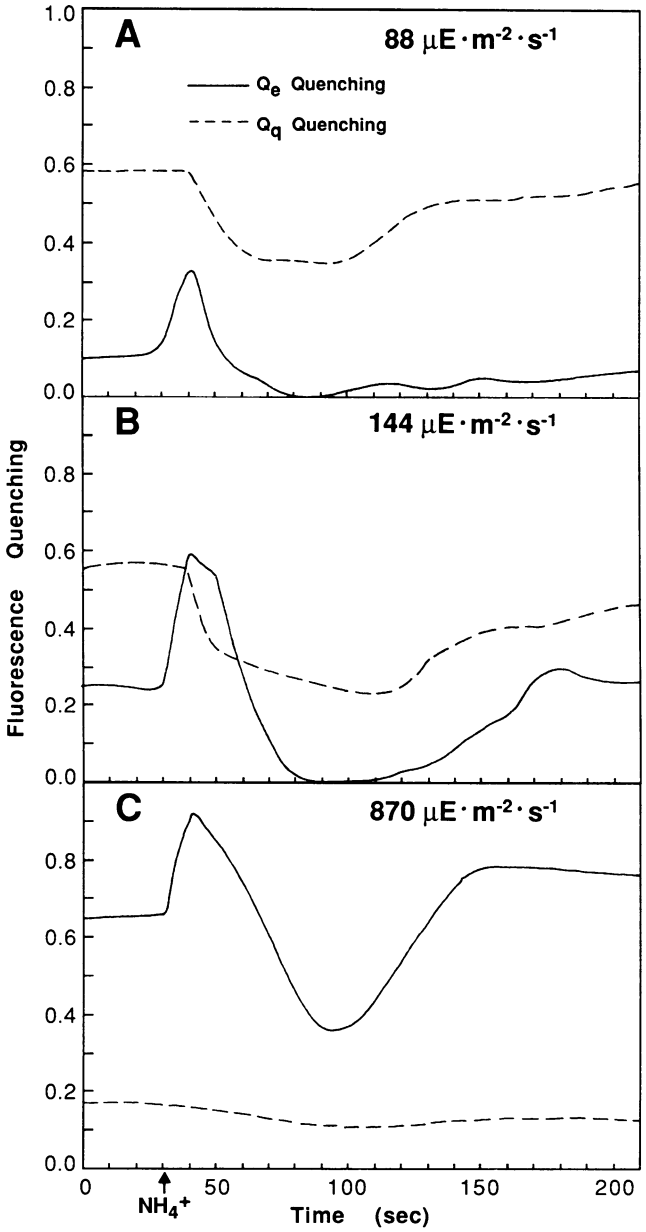

FIG. 2. Effects of ammonium assimilation on fluorescence quenching at three irradiances; A, 88 $\mu E \cdot m^{-2} \cdot s^{-1}$; B, 144 $\mu E \cdot m^{-2} \cdot s^{-1}$; C, 870 uE $\cdot m^{-2} \cdot s^{-1}$. Ammonium was added at a concentration of 100 μM.

DISCUSSION

During steady-state photosynthesis, PSII Chl fluorescence is usually less than maximal due to both photochemical and non-photochemical quenching. Q_q results when high energy electrons from PSII are used to reduce Q. The energy of these electrons, rather than being released as either heat or fluorescence, is used to do work via the electron transport chain. This form of quenching occurs only if oxidized Q is available. Maintenance of a pool of oxidized Q requires the flow of electrons from Q, through PSI, to a physiological electron acceptor such as CO_2, NO_2^- or O_2. Therefore, decreases in fluorescence accompanying an increase in electron flow from quenching are said to be a result of Q_q (10, 14).

The nature of Q_e is not fully understood but is thought to result at least in part from an increase in the thykaloid proton electrochemical potential (14, 19). Establishment of such a potential is thought to favor energy dissipation in the form of heat

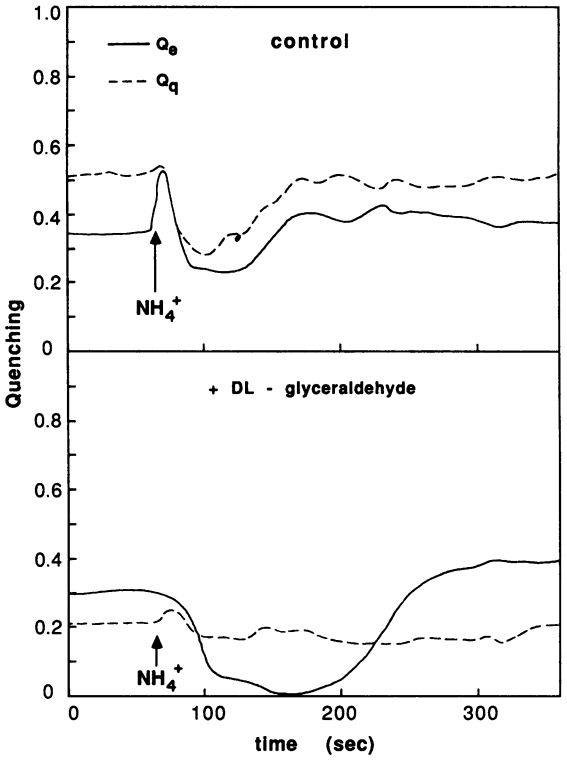

FIG. 3. Effect of ammonium addition to N-limited *S. minutum* on fluorescence quenching in the absence or presence of the Calvin cycle inhibitor D,L-glyceraldehyde.

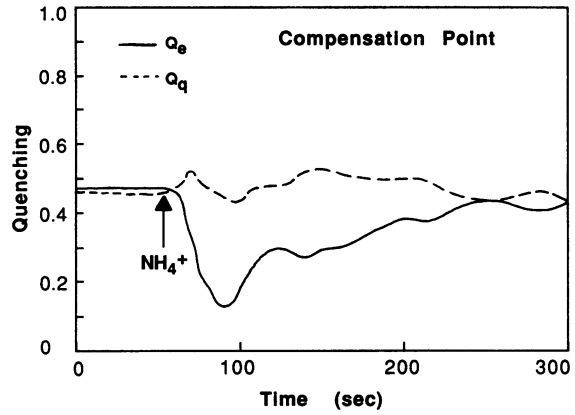

FIG. 4. Effects of ammonium addition on fluorescence quenching in N-limited *S. minutum* held at the CO_2 compensation point.

rather than fluorescence (10, 14, 19). Operationally, Q_e is defined as all quenching other than Q_q.

The interaction between Q_q and Q_e is illustrated by the changes in steady-state quenching that accompany changes in irradiance (Fig. 2). Increases in the photon flux density of white light will decrease the pool of oxidized Q. Consequently, Q_q decreased with increasing irradiance. Correspondingly, increasing irradiance results in increases in the thylakoid proton gradient. As expected, this resulted in an increase in Q_e.

Changes in Quenching during NH_3 Assimilation. The quenching transients which occurred during ammonium assimilation can be broken down into three components based on the order of their occurrence.

Q_e Peak. The Q_e peak designates the rapid increase in Q_e quenching which reaches its maximum approximately 10 s after the addition of ammonium.

Q_q Decline. Approximately 10 to 15 s following ammonium addition, Q_q declines. As would be expected, this coincides with a decrease in O_2 evolution (Fig. 1). The return of Q_q to control levels corresponds with the completion of ammonium assimilation and the recovery of O_2 evolution.

Qe Decline. Approximately 15 to 20 s following ammonium addition, Q_e declines from the Q_e peak and reaches a minimum value at approximately 45 s. Q_e returns to control levels only after ammonium assimilation is complete. The higher the concentration of added ammonium, the longer it takes to be assimilated and, therefore, the longer the declines in Q_q and Q_e.

A potential explanation for these ammonium-induced fluorescence transients is that NH_3 is serving to uncouple photosynthetic electron transport. Several lines of evidence show this is not the case. The first is that the pH (8.0) and the low concentration of added ammonium (100–200 μM) result in concentrations of NH_3 well below that required to uncouple photosynthesis (1). The second is that inhibition of ammonium assimilation into amino acids, by azaserine (6, 18), alleviated all effects of ammonium addition on fluorescence. These results imply that changes in F_v result from the assimilation of ammonium into amino acids and are not due to the presence of NH_3 *per se*. This interpretation is consistent with previous work with this organism, which has shown that these ammonium-induced changes in photosynthetic and respiratory metabolism are due to the assimilation of ammonium into amino acids (3, 6, 7, 18).

The observed changes in fluorescence must therefore be due to the combined effects of a decrease in photodriven electron flow from H_2O to CO_2 and an increase in ammonium assimilation. The first effect of ammonium assimilation on quenching is an increase in Q_e. A possible explanation is a net decrease in ATP consumption resulting in an increase in the thylakoid proton electrochemical potential. This would be consistent with the previously observed decrease in Calvin cycle activity (6) and a resulting decrease in ATP consumption by phosphoribulokinase and phosphoglycerate kinase. Previous work (6, 8) has shown that the decrease in Calvin cycle activity during ammonium assimilation is due in part to RuBP limitation of ribulose bisphosphate carboxylase/oxygenase. Accompanying this decrease in Calvin cycle carboxylation is a decrease in the rate of gross O_2 evolution from water photolysis (18), which corresponds to the decrease in Q_q (Fig. 1). Hence, the Q_q decline may also be attributed to a decrease in Calvin cycle activity or, more specifically, a decrease in carbon flow through glyceraldehyde 3-phosphate dehydrogenase.

Examination of data reported in Figures 1 and 2 show that the Q_e peak always precedes the Q_q decline. If the Q_e peak is due to a decrease in the rate of Calvin cycle kinase reactions and the Q_q decline is due to a decrease in the dehydrogenase reaction, then these results imply that the decrease in carbon flow through the Calvin cycle occurs at the kinases before glyceraldehyde-3-phosphate dehydrogenase.

If the Q_e peak is due to a decrease in ATP consumption by the Calvin cycle then it is apparent this decrease is short-lived. Previous work has shown that although ammonium assimilation takes place at rates of up to 185 μmol·mg^{-1} Chl·h^{-1} the net rate of free amino acid accumulation is only 7.2 μmol·mg^{-1} Chl·h^{-1} (18). This implies that as amino acids become available they are used rapidly (at a rate of approximately 180 μmol·mg^{-1} Chl·h^{-1}) to synthesize cell components. Because 50 to 90% of cell nitrogen in N-limited microalgae is protein (4, 5), a conservative estimate of the rate of protein synthesis would be in the order of 90 μmol·N·mg^{-1} Chl·h^{-1}. Given that 4 ATP are required for the addition of one amino acid to a polypeptide chain, a subsequent increase in ATP consumption of 360 μmol·mg^{-1} Chl·h^{-1} would be expected. As a result, the transit decrease in ATP consumption during initial ammonium assimilation may be

superseded by a large increase in ATP consumption required in support of protein synthesis. This would result in a depolarization of the thylakoid membranes and provide a possible explanation for the Qe decline.

This foregoing model implies that both the Q_e peak and the Q_q decline are due to a decrease in Calvin cycle activity, whereas the Q_e decline is primarily a direct result of nitrogen assimilation. A testable prediction of this model is that ammonium assimilation in the absence of any change in the rate of carbon fixation should result in the disappearance of both the Q_e peak and the Q_q decline, but the Q_e decline should be relatively unaffected. This prediction was tested in two ways. The first involved inhibiting carbon fixation with D,L-glyceraldehyde. Addition of ammonium to these cells resulted in a decline of photosynthetic carbon fixation of only 15 μmol $CO_2 \cdot mg^{-1}$ Chl $\cdot h^{-1}$ (control cells declined by 90 μmol $CO_2 \cdot mg^{-1}$ Chl $\cdot h^{-1}$). During the assimilation of this ammonium, there were only minor changes in Q_q while the Q_e peak was completely eliminated (Fig. 3). Approximately 15 s after the addition of ammonium, the Q_e decline was observed.

The second test of the above prediction follows from work previously undertaken in this laboratory. Birch *et al.* (3) showed that ammonium assimilation by *S. minutum* had no short-term effects on photosynthetic carbon fixation when the cells were at the CO_2 compensation point. As predicted the addition of ammonium under these conditions resulted in little change in Q_q and the absence of the Q_e peak (Fig. 4). Once again, however, the Q_e decline remained.

The results of these experiments imply that the Q_e peak and the Q_q decline are a result of the decrease in Calvin cycle activity during ammonium assimilation. Apparently, the Q_e decline is primarily a result of the assimilation of ammonium, and its occurrence is not dependent upon the accompanying changes in photosynthesis.

CONCLUSION

The results reported in this paper show that the assimilation of ammonium by N-limited *S. minutum* caused dramatic changes in steady-state fluorescence. The transient increase in Q_e and the subsequent decline in Q_q can be attributed to the decrease in Calvin cycle activity which accompanies ammonium assimilation in this organism (6, 8). The decline in Q_e is apparently a direct result of the process of ammonium assimilation. The observation that the Q_e peak always precedes the Q_q decline may reflect a decrease in Calvin cycle carbon flow occurring at the kinase reactions prior to glyceraldehyde-3-phosphate dehydrogenase.

Acknowledgments—We wish to thank Drs. A. G. Miller, R. D. Guy, and G. S. Espie for helpful discussions. A. G. Miller provided helpful advice in the measurement and analysis of fluorescence. Ms. L. Roll aided in manuscript preparation.

LITERATURE CITED

1. Azov Y, JC Goldman 1982 Free ammonia inhibition of algal photosynthesis in intensive cultures. Appl Environ Microbiol 43: 735–735
2. Bassham JA, PO Larsen, AL Lawyer, KL Cornwell 1981 Relationships between nitrogen metabolism and photosynthesis. *In* JD Bewley, *ed.*, Nitrogen and Carbon Metabolism. Junk, London, pp 135–163
3. Birch DG, IR Elrifi, DH Turpin 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. Dortch Q 1982 Effect of growth conditions on accumulation of internal nitrate, ammonium, amino acids and protein in three marine diatoms. J Exp Mar Biol Ecol 61: 243–264
5. Dortch Q, JR Clayton Jr, SS Thoreseu, SI Ahmed 1984 Species differences in accumulation of nitrogen pools in phytoplankton. Mar Biol 81: 237–250
6. Elrifi IR, DH Turpin 1986 Nitrate and ammonium induced photosynthetic suppression in N-limited *Selenastrum minutum*. Plant Physiol 81: 273–279
7. Elrifi IR, DH Turpin 1987 The path of carbon flow during NO_3^--induced photosynthetic suppression in N-limited *Selenastrum minutum*. Plant Physiol 83: 273–279
8. Elrifi IR, JJ Holmes, HG Weger, WP Mayo, DH Turpin 1988 RuBP limitation of photosynthetic carbon fixation during NH_3 assimilation: interactions between photosynthesis, respiration, and ammonium assimilation in N-limited green algae. Plant Physiol 87: 395–401
9. Kanazawa T, MR Kirk, JA Bassham 1970 Regulatory effects of ammonia on carbon metabolism in photosynthesizing *Chlorella pyrenoidosa*. Biochim Biophys Acta 205: 401–408
10. Krause GH, E Weis 1984 Chlorophyll fluorescence as a tool in plant physiology. II. Interpretation of fluorescence signals. Photosynth Res 5: 139–157
11. Mohamed AH, A Gnanam 1979 A possible mechanism of ammonium ion regulation of photosynthetic carbon flow in higher plants. Plant Physiol 64: 263–268
12. Ohmori M, FR Wolf, JA Bassham 1984 *Botryococcus braunii* carbon/nitrogen metabolism as affected by ammonium addition. Arch Microbiol 140: 101–106
13. Schreiber U, U Schliwa, W Bilger 1986 Continuous recording of photochemical and non-photochemical chlorophyll fluorescence quenching with a new type of modulation fluorometer. Photosynth Res 10: 51–62
14. Sivak MN, DN Walker 1985 Chlorophyll *a* fluorescence: Can it shed light on fundamental questions in photosynthetic carbon dioxide fixation? Plant Cell Environ 8: 439–448
15. Strickland JDH, TR Parsons 1972 A practical handbook of seawater analysis. Fisheries Research Board of Canada, Ottawa
16. Turpin DH, IR Elrifi, DG Birch, HG Weger, JJ Holmes 1988 Interactions between photosynthesis, respiration and nitrogen assimilation in microalgae. Can J Bot (in press)
17. Turpin DH 1983 Ammonium induced photosynthetic suppression in ammonium limited *Dunaliella tertiolecta* (Chlorophyta). J Phycol 19: 70–76
18. Weger HG, DG Birch, IR Elrifi, DH Turpin 1988 Ammonium assimilation requires mitochondrial respiration in the light: a study with the green alga *Selenastrum minutum*. Plant Physiol 86: 688–692
19. Weis E, JA Berry 1987 Quantum efficiency of photosystem II in relation to energy-dependent quenching of chlorophyll fluorescence. Biochim Biophys Acta 894: 198–208
20. Wheeler PA 1983 Phytoplankton nitrogen metabolism. *In* ES Carpenter and DH Capone eds, Nitrogen in the Marine Environment. Academic Press, New York, pp 309–346
21. Woo KC, DT Canvin 1980 Effect of ammonia on photosynthetic carbon fixation in isolated spinach leaf cells. Can J Bot 58: 505–510