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Regulation of photosynthetic light harvesting by nitrogen assimilation in the green alga *Selenastrum minutum*

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The interaction of whole cell metabolism with the distribution of excitation energy between photosystem 2 (PS2) and photosystem 1 (PS1), the light state transition, was investigated in vivo in the green alga *Selenastrum minutum*. Nitrogen limited cells of *S. minutum* were presented with a pulse of either NH_4^+ or NO_3^- in the light. As shown previously, CO_2 fixation is inhibited and high rates of N assimilation ensue [(1986) Plant Physiol. 81, 273–279]. NH_4^+ assimilation has a much higher requirement ratio for ATP/NADPH than either CO_2 or NO_3^- assimilation and thus drastically increases the demand for ATP relative to reducing power. Room temperature chlorophyll *a* fluorescence kinetic measurements showed that a reversible non-photochemical quenching of PS2 fluorescence accompanied the assimilation of NH_4^+ but not the assimilation of NO_3^- or CO_2. 77K fluorescence emission spectra taken from samples removed at regular intervals during NH_4^+ assimilation showed that the non-photochemical quenching of PS2 was accompanied by a complementary increase in the fluorescence yield of PS1, characteristic of a transition to state 2. Our data suggests that *S. minutum* responds to the increased demand for ATP/NADPH during NH_4 assimilation by inducing the light state transition to direct more excitation energy to PS1 at the expense of PS2 to increase the production of ATP by cyclic electron transport.

Photosynthesis; State transition; Chlorophyll fluorescence; Nitrogen assimilation; *Selenastrum minutum*

1. INTRODUCTION

In oxygenic photosynthesis the distribution of excitation energy between photosystem 2 (PS2) and photosystem 1 (PS1) is regulated by the light state transition [1,2]. The mechanism of the state transition in green algae and higher plants involves a complementary change in the association of the chl a/b light harvesting complex (LHCII) with PS2 and PS1. Reduction of plastoquinone (PQ) and/or the cytochrome (Cyt) b_6/f complex by excess PS2 activity or chlororespiration [3] in green algae activates a thylakoid membrane-bound kinase which phosphorylates LHCII. The phosphorylation of LHCII is believed to decrease its association with PS2 and increase its association with PS1 via a lateral migration of the phospho-LHCII in the thylakoid membrane. The resulting state is characterized by decreased PS2 activity and increased PS1 activity and is called state 2. Conversely, oxidation of PQ and/or the Cyt b_6/f complex by excess PS1 activity leads to inactivation of the kinase and background phosphatase activity cleaves the phospho-LHCII which then returns to PS2. The re-association of LHCII with PS2 increases PS2 activity at the expense of PS1 activity and defines state 1. The state transition is most often thought of as a regulatory mechanism which serves to optimize the relative activities of PS2 and PS1 under environmental conditions which result in preferential excitation of either PS2 or PS1 [1,2].

It has been shown in intact chloroplasts [4,5] that the state transition is affected by metabolism. In intact maize chloroplasts [4] the degree of phosphorylation observed on transition to state 2 was decreased by the addition of oxaloacetate (which requires reducing equivalents but no ATP) and was increased by pyruvate (which requires ATP but no reducing equivalents). It was suggested by Fernyhough et al. [4] that the demand for NADPH would directly affect the redox state of PQ and thus control the activity of the LHCII kinase as described above. In this way, as suggested by Horton [6], an increased demand for ATP relative to NADPH would trigger LHCII phosphorylation, increased PS1 activity and increased production of ATP via cyclic electron transport.

Horton and Lee [5] have also shown the distribution of excitation energy between PS2 and PS1 (as determined by the degree of Emerson enhancement) to be dependent on the electron acceptor used in intact spinach chloroplasts.

When the green alga *Selenastrum minutum* is cultured under N limitation its capacity for nitrogen assimilation increases [7]. N resupply results in rates of N assimilation into protein which can exceed control rates of photosynthetic carbon fixation. The onset of these rapid rates of N assimilation causes a shut down

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of carbon fixation and the mobilization of starch to provide carbon skeletons for amino acid synthesis [8,9]. The reductant and ATP requirements associated with CO_2, NH_4^+ and NO_3^- assimilation vary and are shown in Table I. The fixation of CO_2 into carbohydrate by the Calvin cycle has an ATP/NADPH consumption ratio of 1.5. The reduction of NO_3^- and its assimilation into protein has an ATP/NADPH consumption ratio of 1.2 while the ATP/NADPH requirement ratio climbs to 5 for the assimilation of NH_4^+ into protein.

A summary of the photosynthetic characteristics of N limited *S. minutum* and the effects of NH_4^+ and NO_3^- assimilation is shown in Table II. The addition of NH_4^+ or NO_3^- to these cells results in high rates of N assimilation [10] which correspond to a decline in the concentration of ribulose bisphosphate (RuBP) below the active site density of ribulosebisphosphate carboxylase (Rubisco) [11]. This RuBP limitation of Rubisco causes a rapid drop in the rate of photosynthetic carbon fixation [11,12]. For NH_4^+ this decline in carbon fixation decreases the demands for photo-generated reductant drastically as illustrated by the decline in gross O_2 evolution. However, there is no change in gross O_2 evolution after addition of NO_3^-, because NO_3^-/NO_2^- replaces CO_2 as the terminal electron acceptor in noncyclic electron flow [12].

The supply of NH_4^+ or NO_3^- to N limited *S. minutum* causes the cells to change from a physiological state characterized by CO_2 fixation to one in which N assimilation is the major metabolic pathway. However, only NH_4^+ assimilation is characterized by a dramatic increase in the requirement for ATP relative to NADPH.

In the present study we exploit the changes in metabolism induced by resupply of NH_4^+ and NO_3^- to investigate metabolic control of the light state transition by the green alga *S. minutum* in vivo. We show that the metabolic demands induced by NH_4^+ assimilation regulate the distribution of excitation energy in photosynthesis to control the production of ATP and NADPH.

2. MATERIALS AND METHODS

Selenastrum minutum (UTEX 2459) was cultured in NO_3^- limited chemostats as previously described [8]. Cells were harvested by centrifugation and used at a final concentration between 4–5 μg chl ml^{-1}. Forty ml of cells were placed in a water-jacketed cuvette for room temperature fluorescence assay.

Room temperature fluorescence emission was monitored continuously during NH_4^+ and NO_3^- assimilation with a PAM fluorimeter (H. Walz Inst., Effeltrich, FRG). The PAM fluorimeter separates the effects of the redox state of PS2 (photochemical quenching) on fluorescence emission from other effects (including state transitions) which contribute to non-photochemical quenching [13]. Frequency sensitive detection ensures that only fluorescence excited by the fluorimeters modulated light source is monitored. The intensity of this source is too low to stimulate PS2 photochemistry and in the absence of additional illumination the minimal fluorescence yield characteristic of open reaction centres (F_o) is determined. Periodically the sample is presented with a brief flash of light intense enough to saturate the photochemistry of PS2. Whilst this light is on, the modulated beam measures the fluorescence level characteristic of closed reaction centres ((F_v)s). The addition of a subsaturating light source will induce variable fluorescence (F_v) which lies between F_o and (F_v)s.

For each experiment cells were initially allowed to dark-adapt for 30 min and F_o and (F_v)s were determined for the dark-adapted cells. Cells were then illuminated with white light from a 300 W projector lamp attenuated to an incident intensity of 78 $\mu E\ m^{-2}\ s^{-1}$ with neutral density filters. F_v induced by this light was monitored continuously and (F_v)s was determined periodically with saturating flashes until stable values were attained. Either NH_4Cl or $NaNO_3$ was subsequently added to a final concentration of 1 mM and F_v and (F_v)s were monitored until the added N was depleted and fluorescence levels were recovered to pre N-addition levels. The cuvette was finally darkened and F_v monitored until a stable level was obtained.

77K fluorescence emission spectra were determined with a home built fluorimeter based on an optical multichannel analyser (EG&G PARC, New Jersey, USA) described previously [14]. Samples (100 μl) were removed from the water-jacketed cuvette at regular intervals during the time course of each experiment, quickly placed into capillary tube sample holders and frozen in liquid nitrogen for later 77K fluorescence emission assay. The averages of 20 scans were taken over the surface of each capillary tube and the relative yields of fluorescence emission between identical samples were accurate to an SE of 4%. Fluorescence yield changes between samples could thus be detected with confidence and normalization of the fluorescence emission spectra to intrinsic or extrinsic fluorescence markers was not required.

3. RESULTS AND DISCUSSION

The distribution of excitation energy between PS2 and PS1 can be estimated by measuring the fluorescence emission from PS2 and PS1. Room temperature fluorescence arises primarily from PS2, however, both PS2 and PS1 fluorescence can be measured at 77K. Room temperature fluorescence emission is sensitive to the amount of excitation energy reaching PS2, the redox state of PS2 and the electrochemical gradient across the thylakoid membrane [15,16]. As described in section 2, we measured room temperature fluorescence emission with a PAM fluorimeter to separate the effects of the redox state of PS2 (photochemical quenching) on fluorescence emission from non-photochemical quenching (which includes state transitions).

Fig. 1 shows the room temperature fluorescence emission trace recorded during the assimilation of NH_4^+. Cells were dark-adapted for 30 min before the F_o determination shown at the start of the trace. At the arrow marked 'light on' the cells were illuminated with 78 $\mu E\ m^{-2}\ s^{-1}$ of white light causing the induction of F_v indicative of the photoreduction of Q_a (the primary quinone electron acceptor of PS2). Determinations of (F_v)s, the light saturated fluorescence yield characteristic of fully reduced Q_a, appear as large spikes superimposed on the F_v trace. $(F_v)s^{dark}$ refers to the light-saturated fluorescence yield of dark-adapted cells determined just prior to illumination.

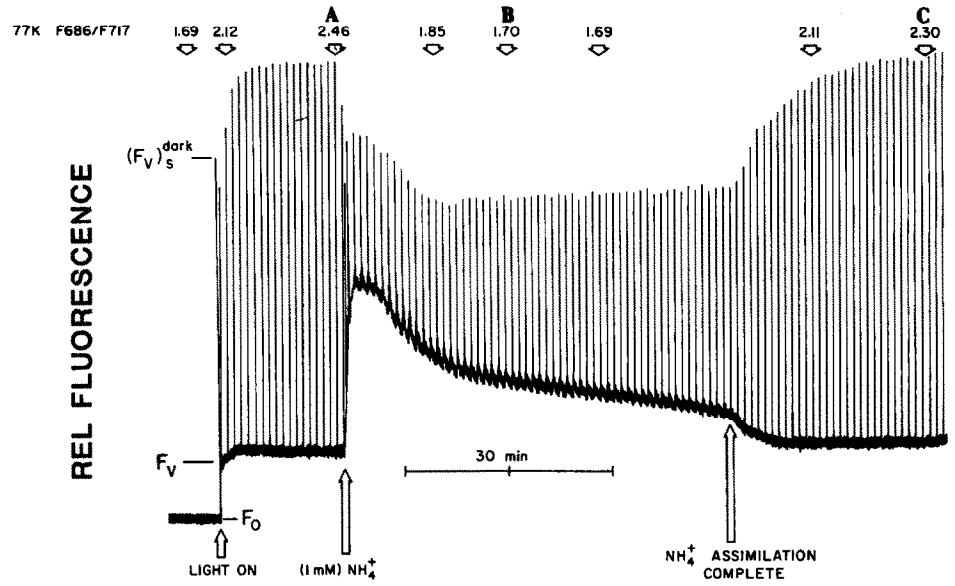

Fig. 1. Room temperature fluorescence induction measured during the time course of NH_4^+ assimilation by N limited *S. minutum* with a PAM fluorimeter as described in the text. Variable fluorescence (F_v) is shown by the continuous trace and the light saturated fluorescence yield (F_v)s is given by the amplitude of the spikes shown on the continuous trace. During the course of this experiment samples were removed periodically for concurrent 77K fluorescence emission determination. The ratio of PS2 to PS1 fluorescence emission at 77K (F686/F717) for each of these samples is noted at the time of sample removal above the PAM trace. The 77K emission spectra of samples A, B and C are shown in Fig. 2b.

As (F_v)s is determined when Q_a is fully reduced, its amplitude is independent of photochemical quenching and is proportional to the amount of excitation energy reaching PS2 minus all non-photochemical quenching processes. (F_v)s was observed to increase in the light over a time period of about 12 min to a level 25% higher than the dark level. This is indicative of either an increase in the amount of excitation energy reaching PS2 or the relief of a dark non-photochemical quenching state. The relative distribution of excitation energy between PS2 and PS1 can be estimated from 77K fluorescence emission spectra [17,18]. Over this same time period the ratio of PS2 (686 nm) to PS1 (717 nm) fluorescence emission at 77K (F686/F717) also increased from a dark value of 1.69 to 2.46 in the light. The 77K data indicated that a change in the amount of excitation energy reaching PS2 was responsible for the increase in (F_v)s. In Fig. 2a the 77K fluorescence emission spectra clearly show that the increase in (F_v)s at room temperature accompanying the dark to light transition was correlated to an increase in PS2 (686 nm, 696 nm) emission and a decrease in PS1 emission (717 nm) at 77K. Such a complementary change in PS2 and PS1 fluorescence emission is characteristic of a state 2 to state 1 transition and confirms earlier reports that green algae [19,20], like cyanobacteria [14], are in state 2 in the dark and convert to state 1 upon illumination.

As reported previously [12], the initiation of NH_4^+ assimilation in Fig. 1 resulted in a rapid increase in F_v accompanied by a rapid oscillation of (F_v)s. The increase in F_v reflects reduction of Q_a, most likely due to a build up of NADPH resulting from the decreased requirement for reductant during NH_4^+ assimilation. The initial oscillation of (F_v)s reflects a transient increase in non-photochemical quenching [12]. Over the next 20 min both F_v and (F_v)s declined at a similar rate. Following this initial decline, F_v continued to slowly decrease while (F_v)s maintained a low steady state until all of the added NH_4^+ had been assimilated. The large

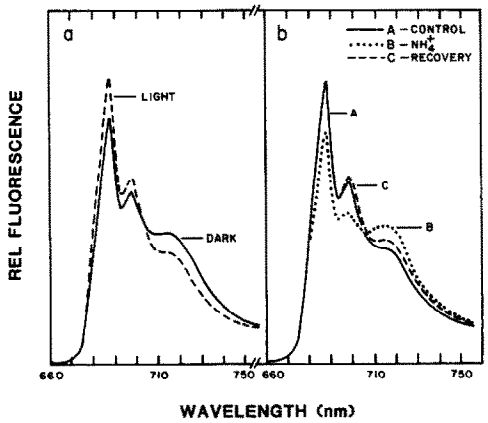

Fig.2. 77K fluorescence emission spectra of *S. minutum*. (a) Cells dark-adapted for 20 min are compared to cells light-adapted (78 μE m^{-2} s^{-1}) for 20 min at room temperature prior to freezing in liquid nitrogen. (b) Fluorescence emission spectra of light-adapted cells before (A), during (B) and after (C) NH_4^+ assimilation. These spectra were obtained from samples taken at the times marked A, B and C in Fig. 1. The excitation wavelength was 435 nm. The spectra have not been normalized.

increase in non-photochemical quenching demonstrated by the decline of (F_v)s was mirrored by a decrease in F686/F717 from 2.46 just prior to addition of NH_4^+ to 1.70 after about 20 min of assimilation. As described above the 77K data indicate that the non-photochemical quenching of PS2 was indicative of a decrease in the amount of excitation energy reaching PS2. After NH_4^+ assimilation was complete F_v and (F_v)s recovered to their pre NH_4^+ levels as did F686/F717. The addition of NH_4^+ does not change fluorescence or metabolism in the presence of the inhibitors of NH_4^+ assimilation, MSX and azaserine [7,10].

The 77K fluorescence spectra of Fig. 2b clearly show that the reversible decrease in (F_v)s induced by NH_4^+ assimilation was accompanied by a reversible decrease in PS2 fluorescence emission (686 nm, 696 nm) and increase in PS1 emission (717 nm). These spectra confirm a complementary change in the distribution of energy between PS2 and PS1 and demonstrate a reversible transition from state 1 during light driven CO_2 assimilation to state 2 during light driven NH_4^+ assimilation.

In the control experiment (not shown), NO_3^- assimilation resulted in relatively small changes in room temperature and 77K fluorescence emission. There was no evidence for a change in the distribution of excitation energy between PS2 and PS1 during NO_3^- assimilation. As discussed earlier both the inhibition of CO_2 fixation and rates of N assimilation have been shown to be very similar for NO_3^- and NH_4^+ but only NH_4^+ assimilation is accompanied by a decline in linear electron flow (oxygen evolution) (Table II).

Our data show that assimilation of NH_4^+ in the light induces a reversible transition to state 2 (an increase in the amount of excitation energy reaching PS1 and decrease of that reaching PS2). This change should stimulate cyclic electron transport around PS1 at the expense of linear electron transport and thus increase the chloroplasts production of ATP relative to NADPH, matching the requirements of NH_4^+ assimilation. The NH_4^+ induced transition to state 2 is likely triggered by the reduction of PQ and/or the Cyt b_6/f complex which results from the decreased requirement for photogenerated reductant during NH_4^+ assimilation. Reduction of the electron transport carriers was demonstrated by the initial increase in F_v induced by NH_4^+. The assimilation of NO_3^- occurs at similar rates to and inhibits CO_2 fixation as much as NH_4^+ assimilation but does not induce state transitions. However, the assimilation of NO_3^- requires photogenerated reductant and therefore does not limit linear electron transport or cause reduction of PQ and/or the Cyt b_6/f complex.

Light-induced state transitions in green algae are probably rare under environmental light conditions as the similarity in the absorption characteristics of the antenna pigments of PS2 and PS1 would make a strong imbalance in energy distribution unlikely.

Table I

The reductant and ATP requirements associated with CO_2, NO_3^- and NH_4^+ assimilation. Reductant requirements (NAD(P)H or ferridoxin) are represented as electron pairs, $2e^-$

Substrate	$2e^-$	ATP	ATP/$2e^-$
CO_2[a]	2	3	1.5
NO_3^-[b]	5	6	1.2
NH_4^+[c]	1	5	5

[a] The fixation of one molecule of CO_2 via ribulose bisphosphate carboxylase produces 2 molecules of 3-phosphoglycerate. The subsequent conversion to triose phosphate requires 2ATP and 2NADPH. Regeneration of ribulose bisphosphate requires an additional ATP

[b] We assume the transport of NO_3^- into the cell requires 1 ATP. Reduction of NO_3^- to NH_4^+ requires 4 NAD(P)H. The subsequent assimilation via glutamine synthetase/glutamine 2 oxoglutarate aminotransferase requires ATP and 2 ferridoxin or one electron pair. Four additional ATP equivalents are required for the addition of an amino acid to a polypeptide chain

[c] We assume NH_3 enters the cell via diffusion. Assimilation of NH_4^+ via glutamine synthetase/glutamine 2 oxoglutarate aminotransferase requires 1 ATP and 2 ferridoxin (one electron pair). Subsequent incorporation of an amino acid into a poly peptide chain requires 4 ATP equivalents. If we assumed NH_4^+ transport into the cell required ATP then the ATP/$2e^-$ ratio would increase from 5 to 6

Our data support the idea of a metabolic control of state transitions and demonstrate this control in vivo under physiological conditions. We show that the state transition is most likely triggered by the redox state of intersystem electron carriers controlled directly by the whole cells downstream requirements for photosynthetic reducing power. Planktonic algae are frequently confronted by nutrient deficiencies in natural systems [21] where nutrient supply can be of a transient nature [22,23]. A transitory supply of nutrients can induce large changes in metabolic demands and, as demonstrated in this work, metabolic regulation of the light state transition can take maximal advantage of a transient nutrient source.

Table II

The effects of N assimilation on photosynthetic metabolism by the N limited green alga *Selenastrum minutum*

Treatment	Rate of N assimilation[a] [9]	Gross CO_2 fixation[a] [11]	Gross O_2 evolution[a] [11]	[RuBP][b] [6,10]	Rubisco active site density[b] [6]
Control	0	125	115	109	60
+ NH_4^+	170	40	60	40	60
+ NO_3	155	35	115	20	60

[a] Values in $\mu mol \cdot mg^{-1}$ chl $\cdot h^{-1}$

[b] Values in $nmol \cdot mg^{-1}$ chl

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REFERENCES

[1] Williams, W.P. and Allen, J.F. (1987) Photosyn. Res. 13, 19–45.
[2] Fork, D.C. and Satoh, K. (1986) Annu. Rev. Plant Physiol. 37, 335–361.
[3] Wollman, F.-A. and Delepelaire, J. (1984) J. Cell Biol. 98, 1–7.
[4] Fernyhough, P., Foyer, C. and Horton, P. (1983) Biochim. Biophys. Acta 725, 155–161.
[5] Horton, P. and Lee, P. (1986) Photosyn. Res. 10, 297–302.
[6] Horton, P. (1987) in: Progress in Photosynthesis Research (Biggins, J. ed.) pp.681–688, Nijhoff, Dordrecht.
[7] Elrifi, I.R. and Turpin, D.H. (1986) Plant Physiol. 81, 273–279.
[8] Elrifi, I.R. and Turpin, D.H. (1987) Plant Physiol. 83, 97–104.
[9] Weger, H.G., Birch, D.G., Elrifi, I.R. and Turpin, D.H. (1988) Plant Physiol. 86, 688–692.
[10] Weger, H.G. and Turpin, D.H. (1989) Plant Physiol. 89, 409–415.
[11] Elrifi, I.R., Holmes, J.J., Weger, H.G., Mayo, W.P. and Turpin, D.H. (1988) Plant Physiol. 87, 395–401.
[12] Holmes, J.J., Weger, H.G. and Turpin, D.H. (1989) Plant Physiol. 91, 331–337.
[13] Schreiber, U., Schliwa, U. and Bilger, W. (1986) Photosyn. Res. 10, 51–62.
[14] Bruce, D., Brimble, S. and Bryant, D.A. (1989) Biochim. Biophys. Acta 974, 66–73.
[15] Krause, G.H. and Weiss, E. (1984) Photosynth. Res. 5, 139–157.
[16] Quick, W.P. and Horton, P. (1984) Proc. R. Soc. Lond. B 220, 371–382.
[17] Butler, W.L. (1978) Annu. Rev. Plant Physiol. 29, 345–378.
[18] Krause, G.H. and Behrend, U. (1983) Biochim. Biophys. Acta 723, 176–181.
[19] Sane, P.V., Furtado, D., Desai, T.S. and Tatake, V.G. (1982) Z. Naturforsch. 37c, 458–463.
[20] Satoh, K. and Fork, D. (1983) Photochem. Photobiol. 37, 429–434.
[21] Dougdale, R.C. (1967) Limnol. Oceanogr. 12, 685–695.
[22] McCarthy, J.J. and Goldman, J.C. (1979) Science 203, 670–672.
[23] Goldman, J.C., McCarthy, J.J. and Peavey, D.G. (1979) Nature 279, 210–215.