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Communication

Evidence for Activation of the Oxidative Pentose Phosphate Pathway during Photosynthetic Assimilation of NO_3^- but Not NH_4^+ by a Green Alga[1]

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ABSTRACT

Addition of NO_3^- to N-limited *Selenastrum minutum* during photosynthesis resulted in an immediate drop in the NADPH/NADP ratio and a slower increase of the NADH/NAD ratio. These changes were accompanied by a rapid decrease in glucose-6-phosphate and increase in 6-phosphogluconate, indicating activation of glucose-6-phosphate dehydrogenase and a role for the oxidation pentose phosphate pathway during photosynthetic NO_3^- assimilation. In contrast, the short-term changes in pyridine nucleotides and metabolites during photosynthetic assimilation of NH_4^+ were not consistent with a stimulation of the oxidative pentose phosphate pathway.

Unicellular algae grown under N limitation respire starch during the assimilation of added NH_4^+ and NO_3^- in both the dark and the light (1, 15, 17, 24). The physiological and biochemical responses to N resupply have been extensively characterized using N-limited *Selenastrum minutum* (19, 20, and references within). Recently, we reported the changes in the pyridine nucleotides and key metabolites in N-limited *S. minutum* upon N resupply in the dark, which indicated that during NH_4^+ assimilation, starch is initially respired to provide carbon skeletons and energy for synthesis of amino acids, whereas the initial requirement of NO_3^- assimilation is electrons to reduce NO_3^- to NH_4^+ (23). Provision of electrons in the dark was facilitated by the production of NADPH via activation of carbon flow through the OPP[3] pathway (23), a pathway that exists in both a chloroplastic and cytosolic form (10, 16).

In light, respiratory CO_2 release is higher during NO_3^- assimilation than during NH_4^+ assimilation (24). Photosynthetic electron transport provides reductive energy for NO_3^- assimilation (11, 12, 18); however, the rate of photosynthetic electron transport has been shown to be insufficient to supply NO_3^- assimilation by N-limited *S. minutum*, and a role for respiration to supply some of this reductive potential has been proposed (24).

We have now examined the pyridine nucleotides and two metabolites, G6P and 6PG, to evaluate the potential role of OPP in providing reducing power to N assimilation by *S. minutum* in the light. Our results suggest that under conditions where the demand for reductive power exceeds that of photosynthetic electron transport, the OPP may be activated in the light to contribute NADPH.

MATERIALS AND METHODS

Cultures of the green alga *Selenastrum minutum* (Naeg.) Collins (UTEX 2459) were maintained under N limitation as previously described (22). Experiments were performed and analyzed as detailed by Vanlerberghe et al. (23) with the following modifications. Cells were concentrated to 5 μg Chl·mL^{-1} and were incubated in continuous light sufficient to saturate photosynthesis (approximately 400 μmol quanta·$m^{-2}\cdot s^{-1}$ at the face of the sampling cuvette) until injection into the appropriate kill solution. After Speed-Vac concentration, samples were brought to 500 μL with distilled H_2O. Samples for G6P and 6PG were killed and extracted as previously described (21). All samples were stored in liquid N_2.

RESULTS AND DISCUSSION

The onset of photosynthetic NH_4^+ assimilation resulted in a 2.5-fold increase in the ratio of NADPH/NADP (Fig. 1, a and b) and NADH/NAD (Fig. 2, a and b). The increase of NADH/NAD was similar to that reported when NH_4^+ is added to N-limited cells in the dark (23). The increase in the NADPH/NADP ratio, however, was larger than that found during experiments in the dark (23), which implicates photosynthetic electron transport as the source of the extra NADPH. Although a transient decrease in O_2 evolution of approximately 50% occurs when NH_4^+ is added to N-limited *S. minutum* (9), there is still significant photosynthetic O_2 evolution to supply electrons for NADP reduction. In addition, photosynthetic carbon fixation, the primary sink for electrons under normal photosynthetic metabolism, is signif-

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[3] Abbreviations: OPP, oxidative pentose phosphate; G6P, glucose-6-phosphate; G6PDH, glucose-6-phosphate dehydrogenase; 6PG, 6-phosphogluconate.

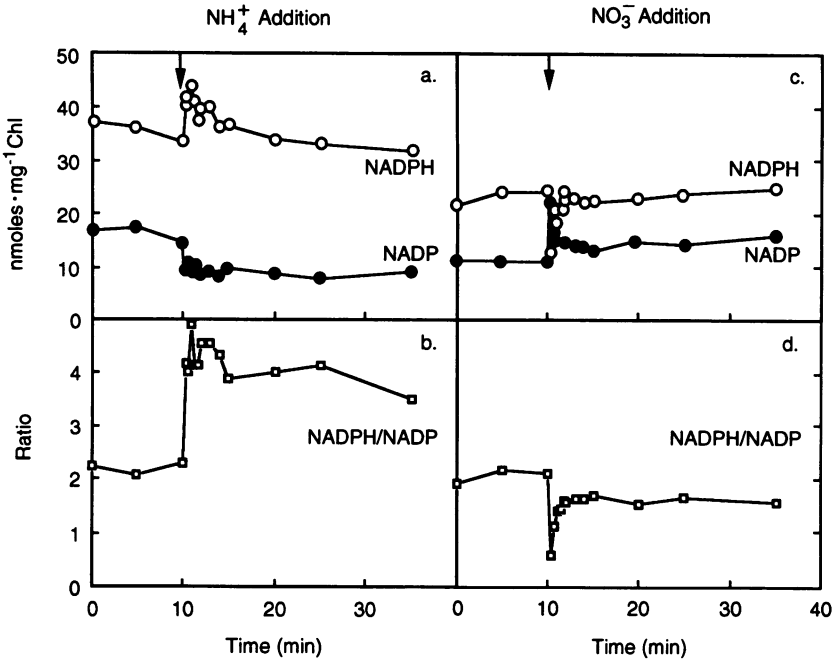

Figure 1. Change of NADPH (○), NADP (●), and the NADPH/NADP (□) ratio at the onset of NH_4^+ (a, b) and NO_3^- (c, d) assimilation by *S. minutum*. The N source was added at the arrow. The data are averages of three experiments.

icantly suppressed during NH_4^+ assimilation by N-limited *S. minutum* (3, 4).

Examination of the G6P and 6PG showed a transient rise in G6P at the onset of NH_4^+ assimilation (Fig. 3a), which is consistent with the induction of starch breakdown, a phenomenon that has been observed upon N resupply to N-limited green algae (1, 15, 17, 24). There was little change in 6PG levels, indicating that carbon flow via the OPP pathway is not stimulated during NH_4^+ assimilation. In fact, the rise in the NADPH/NADP ratio should inhibit the G6PDH, a central enzyme in the OPP pathway (5, 14). The rapid increase in glucose-1-P upon NH_4^+ addition reported previously by our laboratory (15) also indicates that glycolytic degradation of starch predominates during NH_4^+ assimilation.

In contrast, the addition of NO_3^- caused a precipitous drop in the NADPH/NADP ratio (Fig. 1, c and d), a response similar to that observed at the onset of dark NO_3^- assimilation (23). In the light, this ratio recovered to approximately 85% of its steady-state value within 3 min, whereas in dark experiments the ratio had remained low (23). The drop in NADPH was in concert with the onset of NO_3^- reduction and its accompanying demands for Fd and NADPH. Photosynthetic carbon fixation is suppressed by 70% upon resupply

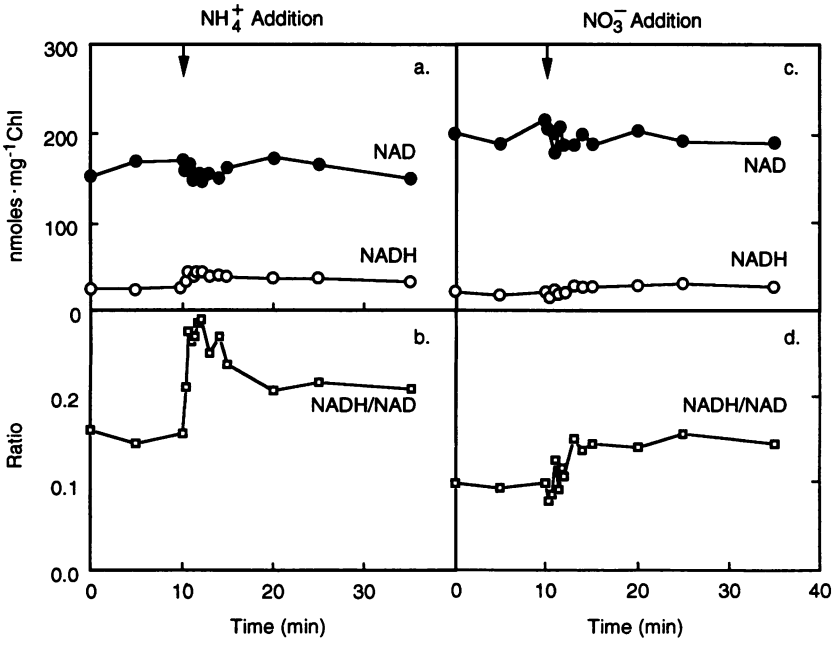

Figure 2. Change of NADH (○), NAD (●), and the NADH/NAD (□) ratio at the onset of NH_4^+ (a, b) and NO_3^- (c, d) assimilation by *S. minutum*. The N source was added at the arrow. The data are averages of three experiments.

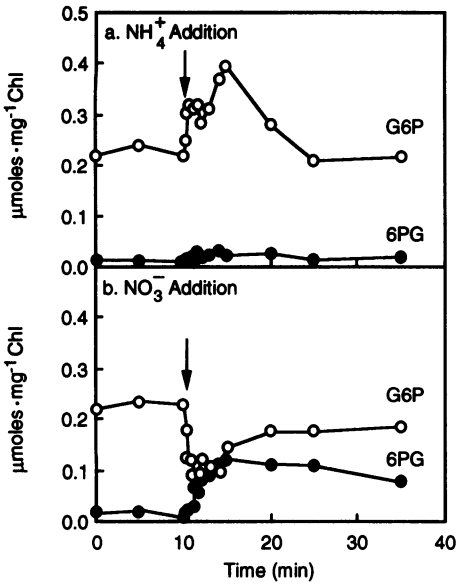

Figure 3. Change of G6P (O) and 6PG (●) levels at the onset of NH_4^+ (a) and NO_3^- (b) assimilation by *S. minutum*. The N source was added at the arrow. The data are averages of three experiments.

of NO_3^- to N-limited *S. minutum* (3, 4), and stable isotope discrimination studies indicate that much of the remaining CO_2 fixation is due to anaplerotic fixation by phospho*enol*-pyruvate carboxylase (7). This shutdown of CO_2 fixation would decrease the demand for NADPH and, thus, may contribute to the partial recovery of the NADPH/NADP ratio in the light.

Measurement of short-term activation changes in the G6PDH activity was not feasible because quantitative extraction of *S. minutum* requires disruption by French press and the redox inactivation of G6PDH is unstable upon exposure to air; however, changes in the G6P and 6PG levels upon addition of NO_3^- are consistent with the activation of carbon flow through OPP (Fig. 3b). Within 1 min of NO_3^- addition, the level of 6PG had increased 5-fold and at 5 min it had reached a maximum level, representing more than a 10-fold increase. This rapid increase, coupled with the decrease in G6P, suggests activation of G6PDH, which controls entrance of carbon into the OPP pathway. Interestingly, G6PDH activity is stimulated by a lowered ratio of NADPH/NADP (5, 14). Starch respiration via the OPP pathway could generate NADPH, supplementing the electron supply from photosynthesis.

During NO_3^- assimilation, the increase of the NADH/NAD ratio was delayed relative to that observed at the onset of NH_4^+ assimilation (Fig. 2, c and d). During dark N assimilation, a similar delay in the change of the NADH/NAD ratio was correlated with a delay in the stimulation of carbon flow through the tricarboxylic acid cycle (23). It appears that, as observed during the dark assimilation (23), the initial demand for carbon respiration upon NO_3^- addition is to reduce NO_3^- to NH_4^+ and only subsequently is carbon flow through the tricarboxylic acid cycle stimulated to meet the demand for carbon skeletons in amino acid synthesis.

These data indicate that the initial requirement of photosynthetic NO_3^- assimilation is reducing power, whereas NH_4^+ assimilation initially requires carbon for amino acid synthesis. The pathways of carbon respiration reflect this difference. During NO_3^- assimilation, at least some starch is respired via the OPP pathway to provide reductive potential, whereas there is no indication that this pathway is activated for NH_4^+ assimilation. Instead, the predominant pathway for starch respiration during NH_4^+ assimilation appears to be glycolytic.

In eukaryotic photosynthetic organisms, the enzymes of the OPP pathway are located in both the cytosol and the chloroplast (10, 16). Attempts to fractionate *S. minutum* by either aqueous or nonaqueous methods have not been successful; therefore, the localization of the metabolite and enzyme activity changes is not known. The major electron sink in the reduction of NO_3^- to NH_4^+ is the nitrite reductase, which is found in the chloroplast of most photosynthetic eukaryotes (2, 6). However, the chloroplastic G6PDH activity in several photosynthetic organisms is thought to be regulated via the Fd/thioredoxin system, which keeps the G6PDH reduced, and, therefore, the enzyme should be inactive in the light (8, 13, 14). The apparent activation of G6PDH during photosynthetic NO_3^- assimilation and the role for redox control in that activation deserves further examination.

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