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# Control of photosynthesis during nitrogen depletion and recovery in a non-nitrogen-fixing cyanobacterium

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**Abstract.** When cells of *Synechocystis* strain PCC 6308 are starved for nitrogen, the amount of stored carbohydrate increases, the phycocyanin to chlorophyll a ratio decreases, and the rates of oxygen evolution and of carbon dioxide fixation decrease. When nitrate-nitrogen is replenished, the amount of carbohydrate decreases, the rate of oxygen evolution increases immediately, preceeding the increase in phycocyanin or carbon dioxide fixation. The rate of respiration first increases and then decreases upon nitrogen addition. Nitrogen-starved cells show no variable fluorescence; variable fluorescence recovered in parallel with oxygen evolution. This suggests that photosystem II is inactive in nitrogen depleted cells and not blocked by a build up of metabolic endproducts. Since carbon dioxide fixation does not increase until two to four hours after nitrate is replenished to nitrogen starved cells, it is suggested that reducing power may first be needed within the cell for some other process than photosynthesis, such as nitrate reduction.

**Key words:** Cyanobacteria – Photosynthesis – Nitrogen depletion – Nitrogen recovery

The relationship between photosynthetic carbon and nitrogen metabolism has been a virtually unexplored aspect of photosynthesis (Romero and Lara 1987). The utilization of nitrate by *Anacystis nidulans* has been considered as a $CO_2$-independent photosynthetic process that makes direct use of photosynthetically generated assimilatory power (Flores et al. 1983), however Romero and Lara (1987) have shown that nitrate utilization can compete with $CO_2$ fixation for assimilatory power when the photosynthetic apparatus is limited by light. A model for the regulation of nitrate uptake involving the products of $CO_2$ assimilation has been presented by Guerrero and Lara (1987), but this is distinct from the influence of nitrogen assimilation on $CO_2$ fixation.

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Although the effect of nitrogen limitation and recovery has been studied in non-nitrogen fixing cyanobacteria in terms of nitrogen-containing macromolecules (i.e. Allen and Smith 1969; Lau et al. 1977; Allen and Hutchison 1980), little information is available about the effect of nitrogen limitation and recovery on photosynthetic rate. Nitrogen starved cells of *A. nidulans* and a mutant of *Agmenellum quadruplicatum* unable to assimilate nitrite were able to use carbohydrate reserves to substitute for $CO_2$ fixation in stimulating nitrate uptake. Ammonia stimulates $CO_2$ fixation by *Anabaena cylindrica* cells and the addition of ammonia to nitrogen-starved cells caused a rapid rise in the incorporation of $^{14}C$ into aspartate and glutamine (Lawrie et al. 1976).

The primary environmental signal for the differentiation of a heterocyst from a vegetative cell is nitrogen limitation (Carr 1983). With nitrogen limitation, all cells mobilize their nitrogen reserves, phycocyanin and cyanophycin granule polypeptide, and $CO_2$ fixation decreases to 10% of that before nitrogen limitation. Studies on the regulation of phycocyanin expression in non heterocystous unicellular cyanobacteria (Lau et al. 1977; Wealand et al. 1989) suggest that nitrogen availability controls phycocyanin synthesis at the transcriptional level. Both metabolic and transcriptional controls, therefore, are involved in nitrogen limitation and repletion.

The present communication reports experiments showing the effect of nitrogen depletion and recovery on photosynthesis in a non-nitrogen fixing cyanobacterium.

## Materials and methods

*Organism and growth conditions. Synechocystis* 6308 (ATCC no. 27150), a unicellular, non-nitrogen fixing cyanobacterium, was routinely grown in liquid medium BG-11 (Allen 1968) supplemented with 2.4 g/l sodium carbonate at 35° C in 10,800 lux of cool white fluorescent light and bubbled with 5% $CO_2$ in air. Low nitrogen conditions and nitrogen repletion conditions were described by Allen and Hutchison (1980). Under low nitrogen conditions cells deplete their supply of nitrogen in 10 h, double twice and then stabilize their $OD_{750}$ at about 1.2 until nitrogen is added back. After nitrogen starvation for 24 h, sodium nitrate or ammonium chloride

was added to 17.6 mM and aliquots were harvested at various hours for analysis.

*Chemical analyses.* Phycocyanin (PC), soluble protein and dry weight analyses were carried out as described previously (Allen and Hutchison 1980). Chlorophyll *a* (Chl *a*) was measured after 80% acetone extraction (MacKinney 1941) of the pellet of a 2 h 40000 rpm ultracentrifugation, in a Ti 65 rotor, of the supernatant of a 15 min 15000 rpm centrifugation in an SS34 rotor, after cells were broken in a French pressure cell at 20000 psi. Cells were concentrated in 10 mM Tris-HCl before breakage. Spectra were run on crude cell extracts on a Perkin Elmer Lambda 5 spectrophotometer. Total carbohydrate was determined by anthrone analysis (Herbert et al. 1971). Cyanophycin granule polypeptide (CGP) was measured in the 15,000 rpm crude extract pellet as previously described (Simon 1973; Allen and Weathers 1980).

### *Photosynthetic parameters*

*Room temperature fluorescence measurements.* Fluorescence induction measurements were made on intact cells suspended in a 2.5 mm tube, the actinic light of 601 nm being provided by a Princeton Applied Research nitrogen pumped dye laser operating at one millijoule per pulse, 10 pulses per second, with 1 ns pulse length. Emission was measured at right angles to the exciting beam through a monochromator at 685 nm using a photomultiplier and a Princeton Applied Research photon counter. Cells were dark adapted for 2 min, and variable fluorescence was measured as the slow kinetic component of the fluorescence induction curve.

*Measurements of oxygen evolution and uptake.* Photosynthetic oxygen evolution and dark respiration were measured at 25° C using a Yellow Springs oxygen electrode. Actinic light was provided by a tungsten-iodine lamp 46 cm from the oxygen electrode, with 3 cm of water between as a heat filter. Respiratory substrates were sodium succinate (3 mM) and sodium ascorbate (5 mM).

*Measurements of carbon dioxide fixation.* $CO_2$ fixation was measured for 15 min at 22—24° C by addition of 5 µCi of 1 mCi/ml of $NaH^{14}CO_3$ (7 mCi/mmol) (New England Nuclear) to 10 ml of magnetically stirred cells in serum stoppered vials in photoflood light (1000 µE $m^{-2}$ $s^{-1}$) using a water filter, in the dark (covered with aluminum foil) or with the addition of DCMU (10 µM) after a 15 min preincubation under the same conditions. Cells were collected on 0.22 or 0.4 µm Millipore filters, washed three times with unlabelled $NaHCO_3$ of the same concentration (0.06 mg/ml), air dried at least three hours and counted by liquid scintillation after addition of Aquasol II. Dry weights were determined in triplicate by filtering the same quantity of cells used for $CO_2$ fixation through washed, dried and tared Whatman GF/C filters and drying to constancy in a 80° C oven.

## Results

Figure 1 shows that as cells became nitrogen starved during 24 h, the photosynthetic rate, measured as oxygen evolution on a chlorophyll basis, and the PC to Chl *a* ratio decreased. The chlorophyll composition remained relatively constant (1.46 to 1.31 µg/ml French pressed cells), but the amount of PC decreased with time of starvation (6.62 to 1.79 µg/ml French pressed cells). This decrease in PC content could also be seen when absorption at 620 nm was compared among crude extract spectra (data not shown). While the oxygen evolution rate was decreasing under conditions of nitrogen starvation, there was no stimulation of the rate by antimycin

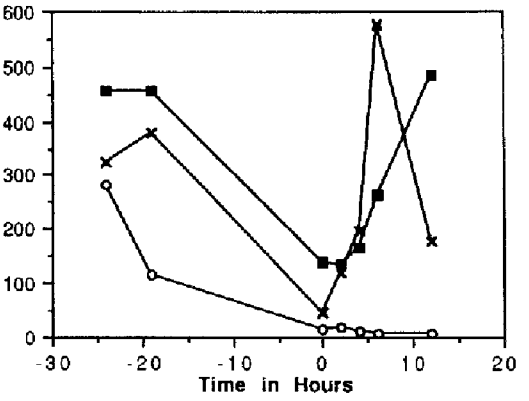


**Fig. 1.** Time course of photosynthetic rate, respiratory rate and PC:chl *a* ratio during 24 h of nitrogen starvation followed by the addition of 1.5 mg/ml sodium nitrate at time 0 after 24 h of nitrogen starvation. ■, µg PC:µg chl *a*; ○, respiratory rate (µmoles oxygen taken up per mg protein per h); ×, photosynthetic rate (µmoles oxygen evolved per mg chl per hour

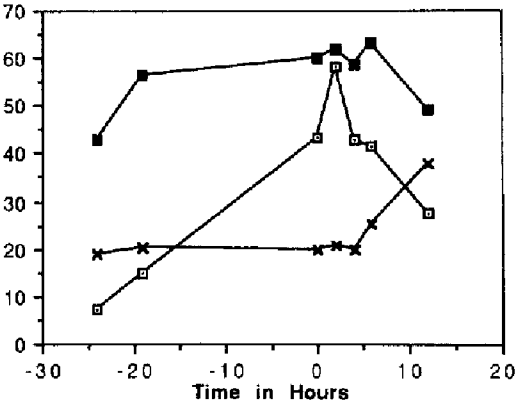


**Fig. 2.** Time course of changes in total carbohydrate and protein in cells starved for nitrogen for 24 h followed by the addition of 1.5 mg/ml sodium nitrate to the culture medium at time 0. □, µg total carbohydrate per µg dry weight; ×, µg protein per µg dry weight; ■, protein to chl *a* ratio

(10 µM), suggesting that the rate of cyclic electron transport around PSI was unaltered.

When nitrogen was added back 24 h after starvation began, the rate of oxygen evolution increased without lag, while the amount of PC did not increase for at least 3 h. Immediately after nitrogen addition a small rise in respiratory rate was seen which paralleled the increase in light-induced oxygen evolution. This respiratory rate increase was not maintained and, in fact, became lower than that found in unstarved cells. The reasons for this are unclear, but may reflect the state of the complex oxygen scavenging systems present in all photosynthetic cells.

As the cells became nitrogen limited, the amount of carbohydrate on a dry weight basis increased until at least 2 h after nitrogen was added back to the culture, increasing from 6% of dry weight to 58% of dry weight in 26 h (Fig. 2). 12 h after nitrogen repletion the cells contained 29% total carbohydrate. As the amount of

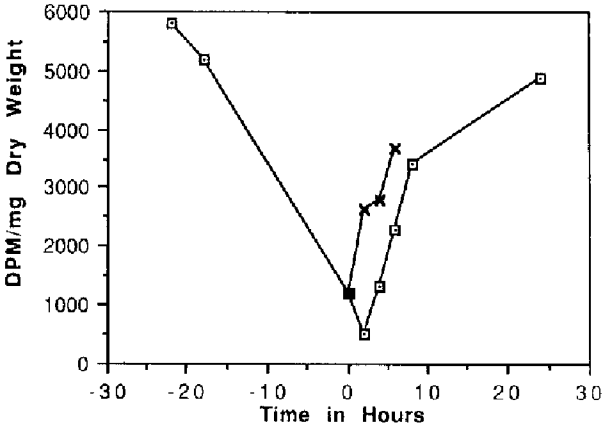


Fig. 3. Carbon dioxide fixation (DPM $NaH^{14}CO_3$ incorporated per mg cellular dry weight) in nitrogen starved and repleting cells. Addition of 17.6 mM nitrogen source was at time 0. □, sodium nitrate; ×, ammonium chloride

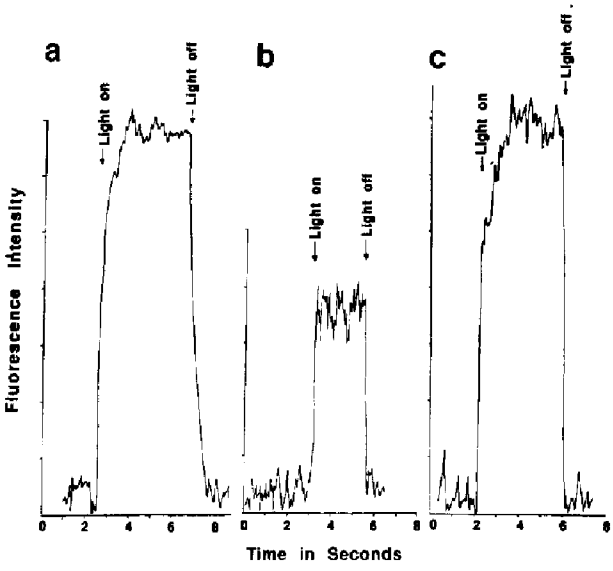


Fig. 4a–c. Fluorescence induction measurements of samples taken during the repletion of nitrogen. Actinic light at 601 nm, emission at 685 nm. All cell suspensions were of the same optical density ($OD_{750}$). Fluorescence intensity in arbitrary units. a Cells grown under full nitrogen supplement; b cells having undergone maximum nitrogen depletion as described in Methods and Materials; c cells 7 h after nitrogen repletion

carbohydrate decreased, the amount of protein in the cells increased, beginning at least 4 h nitrogen was added. The dry weight of the cells increased less than the chlorophyll content increased, as seen when the protein to chl *a* and protein to dry weight ratios are compared at the end of the experiment (Fig. 2).

Figure 3 shows that the rate of $CO_2$ fixation decreased as cells were starved for nitrogen. The rate of $CO_2$ fixation did not begin to increase until 2–4 h after nitrate was added back to nitrogen starved cells. When a more reduced form of nitrogen was added after 24 h of nitrogen starvation, the rate of $CO_2$ fixation increased immediately. Nitrogen starved cells showed no variable fluorescence (Fig. 4). Variable fluorescence recovered in parallel with oxygen evolution, and seven hours after repletion was identical with non-starved cells (Fig. 4). The rate of $O_2$ evolution increased before the significant increase in phycocyanin levels or of $CO_2$ fixation; by 7 h, although $CO_2$ fixation rates and phycocyanin levels were recovering, they had not yet reached normal levels. The correlation between variable fluorescence and oxygen evolution would suggest that PSII is inactive in nitrogen starved cells.

## Discussion

Nitrate is the form of nitrogen which is most widely used by cyanobacteria (Herrero and Guerrero 1986). The effects of nitrogen limitation and recovery on nitrogen-containing macromolecules were followed in *Synechocystis* 6308 by Allen and Hutchison (1980). Removal of nitrogen from the growth medium triggered the degradation of the endogenous nitrogen reserves phycocyanin (PC) and cyanophycin granule polypeptide (CGP). Immediate synthesis of CGP, and protein and PC recoveries 3 h after a utilizable nitrogen source was added, was observed (Allen and Hutchison 1980).

Photosynthetic carbon fixation leads in cyanobacteria to a flow of carbon either to the tricarboxylic acid cycle or to glycogen (Smith 1982). Glycogen can be metabolized via the oxidative pentose phosphate cycle, allowing for cell maintenance in the dark.

After $CO_2$ fixation, the photosynthetic reduction of nitrate to ammonia is quantitatively the most important reductive process in algae (Syrett 1981). The rate of light-dependent $O_2$ evolution by *A. nidulans* cells supplied with saturating concentrations of $NaHCO_3$ was enhanced following the addition of $KNO_3$ to the assay medium (Romero and Lara 1987). This suggested that addition of nitrate, whose reductive assimilation in *A. nidulans* uses electrons directly supplied by photoreduced ferredoxin, would release noncyclic electron flow from a rate limited by the rate of $CO_2$ fixation through the reductive pentose phosphate cycle, thus stimulating the rate of $O_2$ evolution. It was further suggested that nitrate utilization should compete with $CO_2$ fixation for assimilatory power, and that a consequent depression in the rate of $CO_2$ fixation would result. Their data suggest that nitrate-induced depression of $CO_2$ fixation was seen only in limiting light where the nitrate-induced stimulation of non-cyclic electron flow was low. In *Scenedesmus*, no competition between nitrate assimilation and $CO_2$ fixation exists at light saturation (Larsson et al. 1985). Our data with nitrogen-depleted cells indicate depression of $CO_2$ fixation for a number of hours after the addition of nitrate, whereas oxygen evolution increases immediately. These experiments were done in light saturation.

Inhibition of light induced oxygen evolution under conditions of nitrogen depletion suggests that PSII has been rendered inactive. Lack of variable fluorescence, a measure of election transport into the Q pool (Clayton 1969), confirms this. PSII recovers very quickly after nitrate is added back to the cells and prior to a significant increase in phycocyanin levels. Thus, PSII inactivity is

not a consequence of light-harvesting impairment due to phycocyanin deficiency. A reactivation of light-induced oxygen evolution following periods of dark, heterotrophic growth is also rapid in both the cyanobacterium *Chlorogloea fritschii* (Evans et al. 1978) and other eukaryotic algae (Cheniae and Martin 1973). It is noteworthy that a major characteristic of heterocysts is the absence of PSII activity.

Reactivation of PSII following addition of nitrate is not coupled to an increase in $CO_2$ fixation and may therefore be a consequence of the generation of reductant for nitrate reduction, or, possibly, CGP synthesis. That the former explanation is likely, is confirmed by the absence of a lag in $CO_2$ fixation when nitrogen is added back in the form of $NH_4^+$ rather than $NO_3^-$. In this case $CO_2$ fixation recovers over the same timescale as oxygen evolution.

Thus we have demonstrated interaction between $CO_2$ fixation, nitrate reduction and photosynthetic electron transport under conditions of nitrogen repletion of starved *Synechocystis*. The nature of inhibition of PSII, however, has yet to be established.

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