BIOLOGIA PLANTARUM 45 (2): 221-225, 2002

Modulation of the photosynthetic source:sink relationship in cultures of the cyanobacterium *Nostoc rivulare*

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Abstract

Nostoc rivulare was grown in batch cultures under controlled CO_2 and NO_3^- concentrations to modulate the photosynthetic source:sink relationship. Increasing CO_2 supply accelerated the accumulation of chlorophyll (Chl) *a*, *i.e.*, supplemental CO_2 combined with double concentrations of NO_3^- more than doubled the amounts of Chl *a* relative to those of the original medium. Photosynthetic oxygen evolution and respiratory oxygen uptake were both enhanced by elevated CO_2 and NO_3^-. Contents of soluble sugars and starch (total non-structural saccharides) as well as insoluble saccharides (structural fraction) were affected by altering CO_2-NO_3^- combinations. Uptake as well as reduction of either NO_3^- or NO_2^- was inhibited by CO_2 deprivation. Expanding the sink size *via* increasing NO_3^- supply enhanced photosynthesis and thus the sink (NO_3^-) acted as a feed forward stimulator of the source (photosynthesis). The regulatory role of nitrate on photosynthesis was most influential in CO_2-deprived cultures since it could enhance photosynthesis to higher levels than CO_2-supplemented, nitrate-free cultures.

Additional key words: CO_2 enrichment, nitrate, photosynthetic oxygen evolution, respiration, sugars.

Introduction

Nitrogen availability, which is usually in shortage in most habitats worldwide, may limit photosynthesis and/or plant growth. In concomitance with the elevating concentrations of CO_2 in the atmosphere, prospectively expected to accumulate as a result of modern industrialization, higher concentrations of N are needed to cope for accelerated photosynthesis. Otherwise, metabolic disorders (*e.g.*, elevation of saccharide contents at low N) should be expected. Interactions between N assimilation and C metabolism occur through the signal transducer P_{II} (*glnB* gene product) and the global N regulator (*NtcA* gene product). The role of P_{II} may differ in N-fixing and non-N-fixing cyanobacteria (Tandeau de Marsac 2000). Thus, P_{II} is subjected to modifications either under redox control as in the case of *Synechococcus* PCC 7942 and *Synechococcus* PCC 6803 (non-N-fixing) or depends on N-availability (*Anabaena* PCC 7120 and *Calothrix* PCC 7504 (N-fixing). In filamentous heterocystous strains, *Nostoc punctiforme* PCC 73102 and *Calothrix* PCC 7601, no or little modifications have been observed. On the other side, photosynthetic electron transport controls N metabolism in cyanobacteria by means of post-transcriptional modification of *glnB* gene product (Tsinoremas *et al.* 1991).

Depleted N result in limitation of sink development rather than being a direct effect of N supply on photosynthesis (Rogers *et al.* 1998). A prominent feature of high C/N ratio is the "unproductive dry matter", due to the accumulation of photosynthetic products in their primary form as non-structural saccharides (starch and soluble sugars). The accumulation of saccharides exerts feedback inhibition of photosynthesis or even triggers gene expression resulting in lower activities of ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco). Ludewig *et al.* (1998), however, argued against sensing of photosynthesis to sugars since they recorded a positive correlation between starch accumulation and photosynthesis.

The other discrepancy arises from limited or poor CO_2 concentrations, under which many terrestrial (some CAM plants) and aquatic photosynthetic organisms (*e.g.*, cyanobacteria) invented the Carbon Concentrating Mechanism (CCM) to elevate CO_2 concentrations at the

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Received 11 May 2001, *accepted* 8 August 2001.

Abbreviations: CCM - carbon concentrating mechanism; Chl - chlorophyll; Rubisco - ribulose-1,5-bisphosphate carboxylase/oxygenase; TNS - total non-structural saccharides; TSS - total structural saccharides.

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carboxylation site. Carbonic anhydrase, the key enzyme of CCM, catalyzes the dehydration of HCO_3^- to CO_2 (the fundamental substrate for photosynthesis). The impact of bicarbonate, *per se*, on photosynthetic light reactions is a legacy credited back to Otto Warburg (Warburg and Krippahl 1958) and still not yet fully understood. Among several functions speculated, however, Govindjee and his group indicated the necessity of bicarbonate for photosystem 2 stabilisation and water oxidation not least as a proton donor to the plastoquinone site reductase (*e.g.*, Blubaugh and Govindjee 1988). Bicarbonate may be, also, necessary to protect against superoxide radical O_2^- or against photorespiratory formate.

Modulating the source:sink relationship is frequently studied in higher plants (*via* defoliation or cuttings) but much less, if any, in algae or cyanobacteria. For the same purpose we changed the availability of nutrients (CO_2 and NO_3^-) relative to each other as the possible procedure in *Nostoc*. In this work, the cyanobacterium *Nostoc rivulare* was used as the test organism, implying the exclusion of higher plant factors such as nutrient translocation and assimilate allocation. Another difference is that in a cyanobacterial body, the frequency of photosynthesizing cells in relation to gross biomass is much higher than in a higher plant (with non-photosynthesizing tissues such as roots and stems which affect the source:sink relationship). Third, neither the source nor the sink can be completely depleted in this cyanobacterium since fermentation or the oxidative pentose pathway would supply considerable amounts of CO_2 (source) and nitrogen fixation would supply NH^+ (sink).

Materials and methods

Nostoc rivulare (Vaucher) was grown for 10 d at 25 ± 1 °C on the medium of Rippka and Herdman (1993) containing 300 mg(NO_3^-) m^{-3}. Nitrate was supplemented either in the defined concentrations of the original medium (+N) or doubled (+2N) or depleted (-N). Ambient air was bubbled into culture vessels (CO_2), depleted (-CO_2) by air filtration through alkali (10 % NaOH), or supplemented in excess (+CO_2) by passing ambient air on a carbonate buffer which is supposed to supplement 0.33 mol(CO_2) m^{-3} at 25 °C, in addition to the ambient. Therefore, the impact of nine different combinations of CO_2 and NO_3^- was studied in this work:

(1) +CO_2 +2N
(2) +CO_2 +N
(3) +CO_2 –N
(4) CO_2 +2N
(5) CO_2 +N
(6) CO_2 –N
(7) -CO_2 +2N
(8) -CO_2 +N
(9) -CO_2 –N

Content of Chl *a* was determined in hot methanol extracts according to Marker *et al.* (1980). Photosynthetic oxygen evolution in the light and respiratory oxygen uptake in the dark were polarographically determined using a Clark type electrode (*Schott-Geräte*, Hoheim, Germany) under the same irriadiance (5.0 W m^{-2}) and temperature (25 ± 1 °C) as applied for growth. For the determination of saccharides (water soluble and insoluble fractions), the anthrone-sulphuric acid method was used (Fales 1951). Starch content was also determined with the same reagent after being extracted for three times in 0.5 M NaOH by stirring the insoluble fraction mixture for 30 min at 60 °C (Wagner *et al.* 1983). For nitrate uptake and reduction measurements, aliquots of *Nostoc* suspension were provided with 1 M nitrate in the light for 60 min after which the cells were separated from the medium by centrifugation. The cellular (absorbed but not reduced) and medium (not absorbed) nitrate contents were determined after being reduced to nitrite following the method of Kamphake *et al.* (1967) and the nitrites were spectrophotometrically assessed according to Snell and Snell (1949). The data of three replicates were subjected to the one-way analysis of variance using PC-state (University of Georgia, 1985).

Results and discussion

The source:sink relationship refers, in this work, to photosynthesis-nitrogen relationship. In higher plants, however, this concept may extend to include the export or import of photosynthates from one organ to another. In this work, the filamentous, heterocystous, and nitrogen fixing *N. rivulare* was supplemented with various combinations of CO_2 and NO_3^- to expand or limit the size of either the photosynthetic source or sink. Growth was evaluated after 10 d, by Chl *a* content. This exhibited maximum values when the *Nostoc* cultures were supplemented with enriched nutrient concentrations (+CO_2 +2N); lowering the content of either of these nutrients generally decreased Chl *a* content. However, absence of CO_2 was more effective in Chl limitation than absence of NO_3^- (Table 1). Some observations confirm the above statement, *i.e.*, Chl *a* contents were more than 100 % higher in CO_2-supplemented (+CO_2) cultures than that of the ambient (CO_2) although both cultures were provided with equal amounts of nitrate (+2N). In addition, when comparing NO_3^--deprived cultures with NO_3^--doubled ones at any of the given CO_2 concentration Chl contents were not much different.

Photosynthetic oxygen evolution of *N. rivulare* was also affected by the imposed treatments in a trend that is generally similar to, but relatively slower than, that of Chl contents. Nevertheless, a major difference between the response of the two parameters to the imposed treatments was noticed. In CO_2-deprived double-nitrate supplemented cultures ($-CO_2$ +2N), photosynthetic O_2 evolution was higher than that of the ambient CO_2, NO_3^--free culture (CO_2 -N) which contradicts the rational expectation that the higher CO_2 availability, the higher photosynthetic efficiency would be. As well, increasing the sink size (NO_3^- content) significantly accelerated photosynthetic rates at the three different CO_2 concentrations examined (Table 1). Enhancement or limitation of photosynthesis by N sinks confirms the complex network of C and N metabolism up to gene expression. According to Tandeau de Marsac and Lee (1999), this network is coordinated by the gene products cyanate (activator of carbon metabolism) as well as the signal transducer P_{II} protein. The latter controls nitrate/nitrite active transport system to appropriately balance N/C ratio primarily by sensing the availability of ∝-ketoglutarate which is a key regulator for global N control in cyanobacteria and other organisms.

Minimal photosynthetic rates were found, as they should be, in both CO_2 and NO_3^- deprived cultures; where neither a source ($-CO_2$) nor a sink (-N) was externally provided. However, *N. rivulare* is a heterocystous N fixing cyanobacterium implying that a sink might be always existing in the absence of NO_3^-. Alternative sources of CO_2 such as fermentation or the oxidative pentose pathway can be activated at the time that several cyanobacteria can develop the carbon concentrating mechanism (CCM) in case of CO_2 shortage (some of them could evolve CO_2 at concentrations more than sufficient for photosynthesis). For example, the phycoerythrin-possessing cyanobacterium *Synechococcus* sp. WH 783 could evolve CO_2 at rates increasing with irridiance to attain values approximately five fold that of photosynthesis; thus it acts as a source rather than being a sink for CO_2 (Tchernov *et al.* 1997). Taken together, an alternative source:sink relationship could be activated *via* nitrogen fixation and probably CCM in *N. rivulare* cultures that have been deprived of both nitrate and CO_2.

Table 1. Photosynthetic oxygen evolution and respiratory oxygen uptake [mmol(O_2) kg^{-1}(Chl) s^{-1}], unabsorbed NO_3^-, cellular NO_3^- and NO_2^- [g kg^{-1}(Chl *a*)] of *Nostoc rivulare* cultures grown under various combinations of CO_2 and NO_3^-. Significantly different means (at 5 % significance level) are followed by different letter(s).

Treatments		NO_3^- (medium)	NO_3^- (cell)	NO_2^- (cell)	Chl *a*	O_2 evolution	O_2 uptake
$+CO_2$	2N	3.2 b	0.110 c	0.060 c	15.1 a	86.3 a	58.9 a
	+N	1.6 c	0.010 c	0.010 c	11.0 b	75.9 b	53.4 b
	–N	0.0 d	0.003 c	0.000 c	9.6 c	65.8 c	44.6 c
CO_2	2N	3.8 b	0.140 c	0.008 c	7.4 d	57.5 d	41.2 c
	+N	1.9 c	0.009 c	0.002 c	6.5 de	58.3 d	31.2 d
	–N	0.0 d	0.000 d	0.000 c	5.7 ef	39.4 f	29.4 d
$-CO_2$	2N	5.3 a	2.870 a	1.700 a	5.4 ef	47.5 d	29.4 d
	+N	3.2 b	1.430 b	0.800 b	4.9 fg	53.9 d	26.5 d
	–N	0.0 d	0.000 d	0.000 c	3.9 g	32.3 g	18.8 e
$LSD_{0.05}$		0.21	0.16	0.10	1.26	7.66	4.85

Table 2. Soluble saccharides, starch, TNS (total non-structural saccharides), TSS (total structural saccharides) [g kg^{-1}(Chl *a*)] and TNS/TSS, ratios in *Nostoc rivulare* cultures grown under various combinations of CO_2 and NO_3^-. Significantly different means (at 5 % significance level) are followed by different letter(s).

Treatments		Sol. sugars	Starch	TNS	TSS	TNS/TSS
$+CO_2$	2N	1.17 a	1.08 a	2.25 a	1.57 a	1.43
	+N	1.15 ab	0.94 b	2.09 a	1.37 b	1.53
	–N	1.18 a	0.87 b	2.05 ab	1.25 bc	1.64
CO_2	2N	1.16 ab	0.69 c	1.85 bc	1.29 bc	1.43
	+N	1.08 bc	0.66 cd	1.74 cd	1.25 bc	1.39
	–N	1.03 c	0.57 d	1.60 d	1.13 d	1.41
$-CO_2$	2N	0.77 d	0.41 e	1.18 e	0.95 e	1.24
	+N	0.74 d	0.29 f	1.03 e	0.86 ef	1.20
	–N	0.44 e	0.19 g	0.63 f	0.81 f	0.78
$LSD_{0.05}$		0.09	0.09	0.22	0.11	

Respiration of *N. rivulare* was also similarly affected by the modulated nutritional status, *i.e.*, under either of the provided CO_2 concentrations, respiratory oxygen uptake progressively declined as the nitrate concentration was lowered (Table 1). The impact of CO_2 on respiration is demonstrated clearly in these experiments in the sense that at a constant NO_3^- content, respiratory oxygen uptake was enhanced in coincidence with the elevated concentrations of CO_2. However, respiration rates can be sustained essentially unchanged under extremely different nitrate contents by modulating CO_2 concentration (compare treatments 3 with 4 and 6 with 7, each pair gave almost the same respiration rate). Carbon dioxide may or may not affect respiration; internal plant N concentration is particularly involved. In cyanobacteria, the interaction of photosynthesis, respiration, and N metabolism is much more complex not least due to the bioenergetic duality of the electron transport membranes (*e.g.* Peschek 1999) as common sites for both photosynthetic and respiratory electron transport.

The partitioning of photosynthates into structural/nonstructural components was regulated by the availability of CO_2 or NO_3^- (Table 2). In this work, the water soluble saccharide fraction, could be correlated with CO_2 availability; the highest CO_2 level was accompanied with the highest soluble saccharide contents. Starch contents showed a relatively higher response to NO_3^- than soluble saccharides. However, the sum of soluble sugars and starch contents (TNS) exhibited a stronger dependence on NO_3^- and CO_2 combinations; lowering either of the nutrients decreased the values of TNS (a similar response of the insoluble saccharide fraction to treatment combinations was generally obtained). TNS/TSS (total non-structural/total structural saccharides) ratios were proportional to CO_2 concentration. Furthermore, within each concentration of CO_2 this ratio was also affected by NO_3^- shortage: increased at supplemental CO_2, seemingly unaffected at the ambient, lowered at CO_2 deprivation; indicating the removal of soluble sugars by NO_3^-. The accumulation of TNS at elevated CO_2 and N limitation is considered "unproductive dry matter" in terms of carbon balance, on the one hand and impose an impact of accumulated sugars on photosynthesis, on the other hand.

In *N. rivulare*, a positive correlation between TNS and photosynthetic oxygen evolution was generally found. The linearity was highest at supplemental CO_2 (r = 1.000, slope = 37.3) and less at the ambient CO_2 (r = 0.954, slope = 32.4). At deprived CO_2 conditions, however, photosynthetic oxygen evolution lost the positive dependence on TNS (r = 0.81, slope = 27.5) and showed a plateau (or even lowered) with the rise of TNS. Our results, under supplemental or ambient concentrations, are in consistence with those obtained by Ludewig *et al.* (1998) who argued against sensing of photosynthesis to sugars and concluded that the capacity to synthesize leaf Rubisco was not decreased. Under deprived CO_2, however, the inhibitory effect of TNS on photosynthesis seems to occur thus fitting with the interpretation that the accumulation of TNS could inhibit or limit photosynthesis *via* changes in gene expression. Such discrepancies in the impact of TNS on photosynthesis imply the elasticity of metabolism to varied nutritional conditions.

NO_3^- in the medium (representing the "non-absorbed fraction") was generally proportional with the originally supplemented concentrations. These were maximal in the absence of CO_2, followed by the ambient and minimal in $+CO_2$ supplemental cultures confirming that NO_3^- uptake was dependent on CO_2 availability. Cellular NO_3^- content (absorbed but not reduced) was generally very low and insignificantly altered in cultures supplemented with $+CO_2$ or those in the ambient (CO_2) while in CO_2-deprived cultures ($-CO_2$), it was of highest values (Table 1). Therefore, in CO_2-depleted cultures, not only NO_3^- uptake was inhibited but also the absorbed tiny amounts remained in the oxidized form; minimizing in turn the sink size for photosynthates. Also, cellular contents of NO_2^- were very low with insignificant variations among variably treated cultures except in CO_2-depleted cultures these were of highest values. NO_3^- uptake, reduction to NO_2^-, and NO_2^- further reduction (*e.g.* to NH_3) were all inhibited when CO_2 was depleted. Thus, CO_2 acted as a feed-forward stimulator of photosynthesis *via* expanding the sink size (accelerated uptake and reduction of NO_3^-). Furthermore, CO_2 and NO_3^-, instead of competing on photosynthetic electrons, rather enhanced each other.

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