

Glutamine synthetase and arginine inhibition of nitrate reductase activity in *Anabaena cycadeae*

S. Singh* and P.S. Bisen

Wild-type *Anabaena cycadeae* with normal glutamine synthetase (GS) activity utilized arginine as sole N source whereas a mutant strain lacking GS activity did not. Nitrate reductase (NR) activity, higher in the mutant strain than the wild-type strain, was inhibited by arginine though arginine-dependent NH_4^+ generation was higher in the mutant strain than in the wild-type. This suggests that (1) NR activity is NO_3^- -inducible and arginine-repressible; and (2) while GS activity is required for the assimilation of arginine as sole N-source, it is not required for arginine inhibition of NR activity.

Key words: *Anabaena cycadeae*, arginine, cyanobacterium, glutamine synthetase, nitrate reductase.

A better understanding of nitrogen metabolism is a prerequisite for enhancing the potential use of diazotrophic cyanobacteria in biotechnology. Arginine, which accumulates as a reserve material in cyanobacteria, can be utilized as sole N-source by some diazotrophic cyanobacteria (Herrero & Flores 1990). Recently it has been reported that arginine inhibits the nitrate reductase in the diazotrophic cyanobacterium *Anabaena* PCC 7120 (Herrero & Flores 1990). However, the role of glutamine synthetase (GS) activity in arginine assimilation and arginine inhibition of NR activity has not yet been analysed in any cyanobacteria. The role of GS was investigated in the present study using a GS-lacking mutant of *Anabaena cycadeae*.

Materials and Methods

Organisms and Culture Conditions

Anabaena cycadeae and its glutamine auxotroph lacking GS activity were grown as described by Singh (1991). The cultures were incubated at $24 \pm 1^\circ\text{C}$ and illuminated with daylight fluorescent tubes. When needed, arginine (5 mM), KNO_3 (5 mM) and/or NH_4Cl (1 mM) were added to the medium buffered to pH 7.5 with 10 mM HEPES/NaOH. N_2 -fixing cultures of the mutant were obtained by incubating the glutamine-grown cultures in fresh N_2 -medium for 48 h under normal growth conditions.

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Growth Measurements

Growth of the cultures, estimated by measuring the increase in their chlorophyll *a* (chl*a*) content (Mackinney 1941), was expressed in terms of specific growth rate (μ) or the doubling time (t_d).

Estimation of NR Activity

After 12 h in a test medium, the exponentially-growing cells were harvested by centrifugation ($10,000 \times g$, 15 min), washed twice with 50 mM Tris/HCl buffer, pH 7.5, containing NaCl (100 mM), sucrose (300 mM) EDTA (1 mM) and MgCl_2 (5 mM), and resuspended in the same buffer. The suspension was sonicated (25 μm amplitude) for 10 min at 4°C using an ultrasonicator (Soniprep 150; MSE), centrifuged ($20,000 \times g$, 20 min, 4°C) and the supernatant used for measuring NR activity by the method of Manzano *et al.* (1976), following the appearance of NO_2^- (Snell & Snell 1949). The reaction mixture (1 ml) contained 100 mM glycine/KOH buffer, pH 10.5, 20 mM KNO_3 , 4 mM methyl viologen and 10 mM $\text{Na}_2\text{S}_2\text{O}_4$ in 0.1 ml 0.3 M NaHCO_3 and the cell-free extract. The reaction ran for 5 min at 30°C . Cellular protein was estimated following the method of Lowry using BSA as standard.

NH_4^+ Estimation

NH_4^+ in the external medium was estimated by following the colorimetric method of Solorzano (1969).

Results and Discussion

Table 1 shows that the wild-type strain having normal GS activity, grew reasonably well with N_2 , NO_3^- , NH_4^+ or arginine as sole N-source, but the mutant strain, lacking GS

Table 1. Growth, nitrate reductase activity and N₂, NO₃⁻, arginine- and (arginine and NO₃⁻)-dependent NH₄⁺ generation in the wild-type and glutamine auxotrophic mutant strains of *Anabaena cycadeae*.*

Strain	N medium	μ (h ⁻¹)	Nitrate reductase activity (nmol NO ₂ ⁻ (mg protein) ⁻¹ min ⁻¹)	NH ₄ ⁺ in medium (nmol NH ₄ ⁺ (mg protein) ⁻¹ h ⁻¹)
Wild type	N ₂	0.022	0.2	ND
	NO ₃ ⁻	0.024	0.4	ND
	NH ₄ ⁺	0.023	0.05	NE
	Arginine	0.023	0.05	2.0
	Arginine + NO ₃ ⁻	NE	NE	2.0
Mutant	N ₂	0.0	2.0	9.0
	NO ₃ ⁻	0.0	16.0	210.0
	NH ₄ ⁺	0.0	0.3	NE
	Arginine	0.0	0.3	214.0
	Arginine + NO ₃ ⁻	NE	NE	219.0

* Values are means of two independent determinations.

ND–Not detectable; NE–not estimated.

activity, could not grow. Organic and inorganic N compounds could only serve as sole N source in cyanobacteria provided they are metabolized to generate glutamine via GS. Since only the wild-type strain could utilize arginine as sole N source, it seems that GS activity is required for this assimilation. This essentiality of GS becomes understandable only if arginine is catabolized to NH₄⁺ for the GS-dependent production of glutamine.

To determine the role of GS activity in the arginine inhibition of NR activity, NR activity was assayed, both in the wild-type and mutant strains, under various growth conditions (Table 1). The activity in both strains was much higher in NO₃⁻ medium than in N₂-medium, indicating that NR is NO₃⁻-inducible. The activity in both strains was also severely inhibited by arginine and NH₄⁺, indicating that it is under inhibitory control of arginine and NH₄⁺. The persistence of arginine inhibition of NR activity in the mutant strain lacking GS activity indicates that the catalytic function of GS is not required for the inhibition in *A. cycadeae*. As arginine is probably catabolized to NH₄⁺ prior to its assimilation, it is possible that this NH₄⁺ is the inhibitor of NR activity. An NH₄⁺-generating catabolic pathway for arginine would fit with the observation that arginine, like NH₄⁺, caused inhibition of NR activity in both wild-type and mutant strains (Table 1).

To obtain more conclusive evidence regarding the NH₄⁺ generation by arginine prior to its assimilation, the NH₄⁺ in the medium was estimated for both strains in the presence and absence of arginine (Table 1). NH₄⁺ generation was confined to the mutant strain incubated with N₂, NO₃⁻ or arginine. No detectable NH₄⁺ or very low concentrations were generated by identical cultures of the wild-type strain, indicating that the pathway is the main NH₄⁺-assimilating pathway in N₂-fixing cyanobacteria. Furthermore, the level of NH₄⁺ generation by and accumulation in the medium

around the mutant strain with both arginine and NO₃⁻ was similar to that obtained with arginine alone (Table 1). These results elucidate the relative contributions of arginine and NO₃⁻ in NH₄⁺ generation. The NH₄⁺ generated by and accumulated in the medium around the mutant strain in the presence of both arginine and NO₃⁻ is most probably derived from the catabolism of arginine and not by the reduction of NO₃⁻ because the NO₃⁻-reducing system is inhibited by the presence of excess NH₄⁺ (Table 1). NH₄⁺ generation from arginine might account for the capability of the wild-type strain to utilize arginine as sole N source; the growth rate (μ) of the wild-type strain on arginine was similar to those obtained with NO₃⁻ or NH₄⁺ (Table 1). NH₄⁺ generation can thus be used to determine the rates at which arginine catabolism and NO₃⁻ reduction proceed.

In conclusion: (1) NR activity in *A. cycadeae* is NO₃⁻-inducible and arginine-repressible; (2) NH₄⁺ from the catabolism of arginine may be the inhibitor of NR activity; and (3) while GS activity is required for the assimilation of arginine, it is not required for the arginine inhibition of NR activity.

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