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Nitrogen nutrition in the cyanobacterium *Nostoc* ANTH, a symbiotic isolate from *Anthoceros*: Uptake and assimilation of inorganic-N and amino acids

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Amino acid uptake and utilization of various nitrogen sources (amino acids, nitrite, nitrate and ammonia) were studied in *Nostoc* ANTH and its mutant (Het^-Nif^-) isolate defective in heterocyst formation and N_2-fixation. Both parent and its mutant grew at the expense of glutamine, asparagine and arginine as a source of fixed-nitrogen. Growth was better in glutamine-and asparagine-media as compared to that in arginine media. Glutamine and asparagine repressed heterocyst formation, N_2-fixation and nitrate reduction in *Nostoc* ANTH, but arginine did so only partially. The poor growth in arginine-medium was not due to poor uptake rates, since the uptake rates were not significantly different from those for glutamine or asparagine. The glutamine synthetase activity remained unaffected during cultivation in media containing any one of the three amino acids tested. The uptake of amino acids was substrate-inducible, energy-dependent and required *de novo* protein synthesis. Nitrate and ammonium repressed ammonium uptake, but did not repress uptake of amino acids. In N_2-medium (BG-11_0), the uptake of ammonium and amino acids in the mutant was significantly higher than its parent strain. This was apparently due to nitrogen limitation since the mutant was unable to fix N_2 and the growth medium lacked combined-N.

Most N_2-fixing cyanobacteria are able to metabolize a wide range of combined nitrogen sources, the most common of these being ammonia and nitrate. Some of them are also capable of utilizing amino acids, such as glutamine, arginine and asparagine as sole nitrogen source[1-3]. However, the relative efficiency of such amino acids supporting cyanobacterial growth varies greatly. *Synechococcus* PCC 6803 grows as well on arginine as on nitrate, but *Anabaena* sp. PCC 7120 grows more slowly on arginine than on nitrate. It has been suggested that both high and low affinity arginine transport systems contribute to arginine–dependent growth in *Anabaena* sp. PCC 7120[1,4].

Inducible as well as repressible-derepressible processes are known to be involved in the regulation of cyanobacterial nitrogen assimilation. Most studies in the past have been confined to inorganic nitrogen regulation of N_2-fixation, and of uptake and assimilation of nitrate, nitrite and ammonia[2,3]. However, the information concerning amino acid nitrogen nutrition, nitrogen regulation of amino acid uptake, and the associated metabolic changes is scanty, as well as contradictory and therefore, requires further study[1,5-9]. Available reports[1] indicate that arginine is a weak repressor of nitrogenase and nitrate reductase in *Anabaena* sp. PCC 7120. Conversely, nitrate reductase activity is induced by arginine in *Oscillatoria chalybea*[10]. Similarly, the effect of glutamine was found to be variable on heterocyst formation and nitrogenase activity in *Anabaena variabilis*; it repressed nitrogenase activity, but not heterocyst formation[6].

Nostoc ANTH utilizes nitrate, nitrite and ammonia as nitrogen sources, but has not been characterized with regard to amino acids uptake and nutrition. To study the latter aspects in this cyanobacterium, a mutant (Het^-Nif^-) defective in heterocyst formation and N_2-fixation was isolated. Amino acids nitrogen nutrition, the associated metabolic changes and amino acid uptake was compared in this mutant and its parent strain (*Nostoc* ANTH). Our results indicate that glutamine and asparagine served as better sources of nitrogen for growth, followed by arginine The formation of heterocysts, and nitrogenase and nitrate reductase activities were completely repressed by glutamine and asparagine, but only partially by arginine. The glutamine synthetase activity, however, remained unaffected in the presence of these amino acids. Glutamine, asparagine, arginine, and ammonium uptake activities were higher in the mutant than in its parent strain. The uptake of these amino acids was biphasic, energy-dependent and required *de novo* protein synthesis. Unlike ammonium uptake, the

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glutamine, asparagine and arginine uptakes were not repressed by nitrate and ammonium.

Materials and Methods

Strains and culture conditions

Axenic cultures of the diazotrophic cyanobacterium *Nostoc* ANTH and its Het$^-$Nif$^-$ mutant were grown in batch cultures using BG11$_0$ (N_2-medium) supplemented with 5 m*M* KNO_3 (NO_3-medium), at 25°C with a photon fluence rate of 50 μmol. m^{-2}. s^{-1} (ref. 11). When needed, the N_2-medium was supplemented with 2 m*M* NH_4Cl (NH_4^+-medium), or 1 m*M* L-glutamine, asparagine or arginine. The medium was buffered with equimolar concentration of HEPES and its *p*H was adjusted to 7.5 before autoclaving.

Isolation of the mutant

The Het$^-$Nif$^-$ mutant was isolated from nitrosoguanidine (NTG) mutagenized cultures of *Nostoc* ANTH. Exponentially growing cultures were sonicated (MSE Soniprep) to yield short filaments (approx. 3-4 cells per filament) and treated with 100 μg.ml^{-1} of NTG for 90 min in 10 m*M* citrate buffer (*p*H 6). The treatment resulted in 95% killing. The mutagenized cultures were washed and incubated in NH_4^+-medium for 4 days to permit segregation and expression of mutations. These NH_4^+-grown cells were washed and incubated for 2 days in N_2-medium (BG-11$_0$) and subsequently treated with 60 μg.ml^{-1} of penicillin for 24 hr to kill N_2-fixing cells. The survivors (5.6×10^5 colony-forming units) were plated on solid N_2-medium containing 100 μ*M* KNO_3. Colonies appeared after one week of incubation. The colonies that were unable to grow and produce heterocysts and lacked nitrogenase activity in N_2-medium, i.e., the Het$^-$Nif$^-$ mutants, arose at a frequency of 4.1×10^{-4}. One such mutant was further characterized with regard to uptake and assimilation of ammonium and amino acids.

Growth, heterocyst frequency and nitrogenase activity

Growth was measured as increase in concentration of Chl *a*[12]. Heterocyst frequency was calculated as percentage of total cells by light microscopic observations after 72 hr of incubation in different nitrogen media. Acetylene reduction assay was used to measure nitrogenase activity[13].

Glutamine synthetase (transferase) and nitrate reductase activities

These activities were measured in alkyltrimethylammoniumbromide (CTAB) permeabilized cells. Glutamine synthetase activity was measured as described by Sampaio *et al.*[14]. Ferredoxin-dependent nitrate reductase activity was measured using dithionite-reduced methyl viologen as reductant[15]. Nitrite was estimated colorimetrically as described by Snell and Snell[16]. Protein concentration was measured according to Lowry *et al.*[17].

Ammonium and amino acid transport assays

Ammonium transport assay was done using the radioactive analogue of ammonium, [^{14}C]-methylammonium (sp. activity 172 kBq.μmol^{-1})[18]. Glutamine, arginine and asparagine uptakes were measured using ^{14}C labelled glutamine (sp. activity 256 kBq.μmol^{-1}), arginine (sp. activity 65 kBq.μmol^{-1}) and asparagine (sp. activity 63 kBq.μmol^{-1})[8]. The NO_3-grown cultures of the parent and its Het$^-$Nif$^-$ mutant were harvested during the exponential growth phase, washed in N_2-medium and then incubated in N_2-medium, NO_3-medium and in N_2-medium supplemented with 1 m*M* glutamine, arginine or asparagine for 48 hr. The mutant was incubated for only 24 hr in N_2-medium, because it lacked growth in N_2-medium. After incubation in different nitrogen-media the cells were harvested, washed and resuspended in 10 m*M* HEPES-NaOH buffer (*p*H 7.0) to a concentration of 5 μg Chl *a*.ml^{-1}. The ^{14}C labelled methylammonium, glutamine, arginine and asparagine were added to a final concentration of 50 μ*M*. The uptake experiments were carried out at 25°C and a photon fluence rate of 50 μmol.m^{-2}.s^{-1}. Wherever needed, dicyclohexylcarbodi-imide (DCCD, 50 μ*M*), dichlorophenyl dimethylurea (DCMU, 10 μ*M*) and carbonyl cyanide chlorophenyl hydrazone (CCCP, 25 μ*M*) were added to the cell suspension 30 min prior to the addition of labelled amino acids and were present during the experiments. At different time intervals, 400 μl samples were taken out rapidly and the cells were separated from their bathing medium by centrifugation through silicon oil DC 550/dinonyl phthalate (40/60, v/v) into perchloric acid/water (15/85, v/v)[18]. The ^{14}C in perchloric acid fraction was measured using a liquid scintillation counter (Model 1801, Beckman Instruments). Non-specific binding of [^{14}C]-methylammonium, glutamine, asparagine or arginine was determined by measuring its incorporation in toluene-treated cells[18]. This value was always subtracted from the value obtained for toluene-treated cells.

Results and Discussion

As shown in Table 1, *Nostoc* ANTH grew well in N_2-medium with KNO_3 and NH_4Cl proving equally

good sources of nitrogen for the growth. The growth of the Het⁻Nif⁻ mutant in nitrate and ammonium-media was similar to its parent strain; however, it did not grow in N_2-medium. The mutant lacked heterocysts and did not exhibit any nitrogenase activity and therefore, could not utilize N_2. Furthermore, nitrogenase activity in the mutant remained undetectable, even under anaerobic conditions indicating that it did not possess the second nitrogenase (Nif 2) activity reported to be expressed in *Anabaena variabilis* under anaerobic conditions[19,20]. The Het⁻Nif⁻ mutant provided us an experimental system to assess the ability of different amino acids to serve as sole nitrogen source for growth of *Nostoc* ANTH.

Out of the 20 amino acids tested, only glutamine, asparagine and arginine supported the growth of the Het⁻Nif⁻ mutant (Table 1). The growth was similar in the parent and its mutant in media supplemented with asparagine or glutamine. However, the growth of the parent strain was lower in arginine-supplemented medium, and in case of the mutant, it decreased even further. As in nitrate and ammonium media, heterocyst formation and nitrogenase activity in the parent strain was repressed in glutamine- and asparagine-supplemented media. In contrast, there was only a partial repression in the arginine-grown parent strain. This is in agreement with similar observations with *Anabaena* PCC 7120[1]. These observations may also explain a better growth rate of the parent strain than its mutant in arginine-supplemented medium as the parent could still use N_2, at least partly, in presence of arginine. These results indicate that arginine was not as good a nitrogen source as glutamine and asparagine for *Nostoc* ANTH. Our results agree with the glutamine-mediated inhibition of nitrogenase observed in *Anabaena variabilis*, but in contrast to *A. variabilis*, glutamine also inhibited the heterocyst formation in *Nostoc* ANTH[6].

The nitrate reductase and glutamine synthetase activities of the Het⁻Nif⁻ mutant were similar to its parent strain (Table 2). The nitrate reductase activity in both the parent and its mutant strain was nitrate inducible/ammonium repressible. This activity was also repressed in the glutamine- and asparagine-grown cultures, whereas only a partial repression (approx. 50%) was observed in presence of arginine. Thus, glutamine and asparagine strongly inhibited nitrate reductase activity, whereas arginine did so only partially. These results are in agreement with partial repression of nitrate reductase activity by arginine in *Anabaena* PCCC 7120[1] and in contrast to the arginine induction of nitrate reductase activity in *Oscillatoria chalybea*[10]. The glutamine synthetase activity was partially repressed in ammonium-grown parent and its mutant strain; however, no significant repression of activity was observed in either strains in presence of glutamine, asparagine and arginine (i.e., the activity remained nearly similar to those in N_2-grown cultures) as reported earlier in *Nostoc muscorum*[8].

Ammonium transport system has been characterized in *Nostoc* ANTH using [^{14}C]-methylammonium and found to be ammonium repressible[21]. In the present study, the N_2-grown parent showed ammonium uptake rate of 7 nmol/min/mg Chl *a*. However, the rate of ammonium uptake in the mutant was highly derepressed, being nearly two-fold higher than that in its parent strain. Since the mutant can not use N_2 as nitrogen source, incubation of the mutant in N_2-medium must have led

Table 1—Growth, heterocyst frequency and nitrogenase activity of *Nostoc* ANTH and its mutant as a function of nitrogen source

[4 day old nitrate-grown cultures were used as initial source of inoculum. The growth (μg Chl *a*/ml), frequency of heterocysts (per 100 vegetative cells) and aerobic nitrogenase activity (nmol C_2H_4 formed/μg Chl *a*/h) were determined after 72 hr of growth in BG-11_0 media containing different nitrogen sources as indicated. Chl *a* concentration at the start of incubation (0 time) was 0.29 μg Chl *a*/ml. Values presented are means ± SE from two independent experiments, each with two replicates]

Addition to BG11_0 (m*M*, in parenthesis)	Parent			Het⁻Nif⁻ mutant		
	Growth	HF	N_2ase	Growth	HF	N_2ase
Nil	1.2 ± 0.1	5.3 ± 0.2	12 ± 0.6	0.1 ± 0.0	0.0	0.0
NO_3^- (5)	1.3 ± 0.1	0.0	0.0	1.2 ± 0.1	0.0	0.0
NH_4^+ (2)	1.4 ± 0.1	0.0	0.0	1.5 ± 0.1	0.0	0.0
Glutamine (1)	2.1 ± 0.2	0.2	0.1	2.0 ± 0.2	0.0	0.0
Asparagine (1)	1.9 ± 0.2	0.3 ± 0.1	0.5 ± 0.1	1.7 ± 0.1	0.0	0.0
Arginine (1)	1.2 ± 0.1	2.6 ± 0.3	6.1 ± 0.3	0.9 ± 0.1	0.0	0.0

HF, heterocyst frequency; N_2ase, nitrogenase activity

Table 2—Nitrate reductase and glutamine synthetase transferase activities of *Nostoc* ANTH and its Het^-Nif^- mutant as a function of nitrogen source

[4 day old nitrate-grown cultures were used as initial source of inoculum. Nitrate reductase (NR) activity (nmol NO_2^- formed/min/mg protein) and glutamine synthetase (GS) transferase activity (nmol γ-glutamyl hydroxamate formed/min/mg protein) were determined after 24 hr of incubation in BG-11_0 media containing different nitrogen sources as indicated. Chl *a* concentration at the start of incubation was 4 μg Chl *a*/ml. Values presented are means ± SE from two independent experiments, each with two replicates]

Addition to BG-11_0 (m*M*, in parenthesis)	Parent		Het^-Nif^- mutant	
	NR	GS	NR	GS
Nil	1.8 ± 0.11	610 ± 7	2.1 ± 0.03	648 ± 4
NO_3^- (5)	4.2 ± 0.15	598 ± 3	4.8 ± 0.11	593 ± 7
NH_4^+ (2)	0.2 ± 0.10	376 ± 6	0.4 ± 0.18	371 ± 8
Glutamine (1)	0.3 ± 0.06	595 ± 8	0.5 ± 0.10	582 ± 4
Asparagine (1)	0.5 ± 0.04	581 ± 7	0.4 ± 0.16	594 ± 6
Arginine (1)	0.9 ± 0.01	601 ± 4	1.1 ± 0.02	598 ± 2

Table 3—[^{14}C]-Methylammonium uptake activity in *Nostoc* ANTH and its Het^-Nif^- mutant grown in different nitrogen media

[Nitrate-grown exponential cultures were washed and transferred to N_2-, NO_3^-- or NH_4^+-media and incubated for 48 hr. The cells were then washed, resuspended in HEPES buffer and used for [^{14}C]-methylammonium uptake as described in Materials and Methods. The N_2-grown cells in case of Het^-Nif^- mutant refer to the nitrate-grown cells, which were subjected to nitrogen starvation for 24 hr in N_2-medium; this is because the mutant does not grow in N_2-medium. The uptake rates were calculated from the linear phase between 2 and 10 min after [^{14}C]-methylammonium addition. The uptake rates are expressed as nmol/min/mg Chl *a*. Values presented are means ± SE from two independent experiments, each with two replicates]

Growth medium	[^{14}C]-Methylammonium uptake	
	Parent	Het^-Nif^- mutant
N_2-medium (BG11_0)	7 ± 0.48	13 ± 0.12
NO_3^--medium (BG11_0+5m*M* KNO_3)	0.6 ± 0.07	0.7 ± 0.01
NH_4^+-medium (BG-11_0+2m*M* NH_4Cl)	0.3 ± 0.03	0.2 ± 0.06

to nitrogen starvation and therefore, the high rate of ammonium transport activity. The ammonium uptake activity was repressed in nitrate or ammonium-grown cultures of both the parent and the mutant strains (Table 3). Overall, the data show that both ammonium and nitrate repress the ammonium transport activity and that nitrogen starvation leads to increased ammonium transport activity.

Since glutamine, asparagine and arginine were utilized as nitrogen sources for growth by *Nostoc* ANTH and its Het^-Nif^- mutant, uptake of such amino acids were studied as a function of different nitrogen sources in the media. Glutamine uptake was biphasic in nature in both the parent and its mutant strains grown in N_2- and glutamine-media (Fig. 1). The initial rapid phase lasted for less than 2 min followed by a slower second phase during the remaining experimental period. The rates of glutamine uptake were higher in the mutant than that in the parent strain. The N_2-grown parent strain showed glutamine uptake rates of 55 and 11 nmol/min/mg Chl a, and the N_2-grown Het^-Nif^- mutant 70 and 27 nmol/min/mg Chl a, during the first and second phase, respectively. In glutamine-grown cultures, the observed glutamine uptake rates in parent were 93 and 44 nmol/min/mg Chl *a* and in mutant 95 and 45 nmol/min/mg Chl *a*, during first and second phase, respectively. Thus, the glutamine uptake rates were higher in glutamine-grown cultures than in N_2-grown cultures. This increase in the rates was significantly inhibited by chloramphenicol, an inhibitor of protein synthesis (Fig. 1). The activity and pattern of glutamine uptake in nitrate- and ammonium-grown cells of both the parent and its mutant strain was similar to that in N_2-grown cells. Chloramphenicol had a similar effect on asparagine and arginine uptakes also (data not shown).

The N_2-grown parent strain showed asparagine uptake rates of 30 and 11 nmol/min/mg Chl *a*, and the Het^-Nif^- mutant exhibited uptake rates of 78 and 33 nmol/min/mg Chl a, during first and second phase, respectively. The rates of asparagine uptake increased in the asparagine- grown parent strain to 56 and 29.2

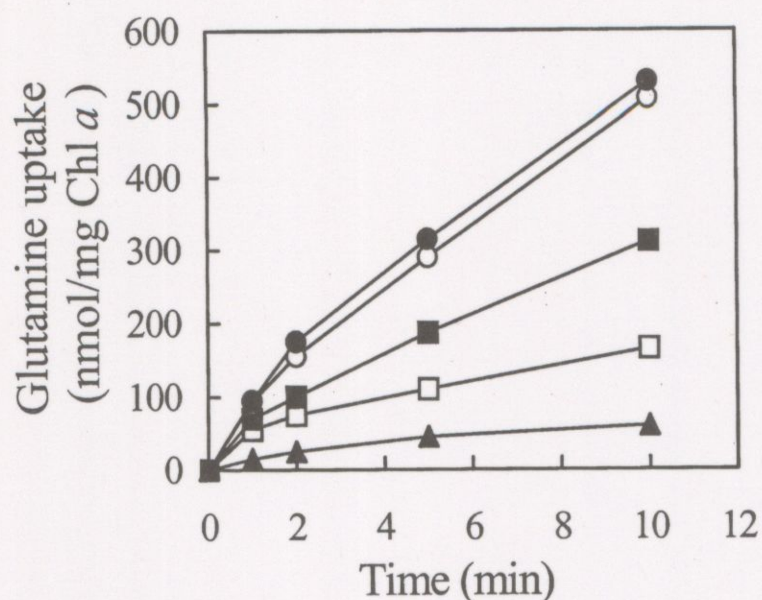

Fig. 1—[^{14}C]-Glutamine uptake in *Nostoc* ANTH and its Het⁻Nif⁻ mutant [Nitrate-grown exponential cultures were washed and transferred to N_2- ($BG11_0$) and glutamine- ($BG11_0$+1 m*M* glutamine) media and incubated for 48 hr. To one set of glutamine-medium chloramphenicol (1 μg/ml) was added at the beginning of incubation. The N_2-grown cells in case of mutant refer to the nitrate-grown cells, which were subjected to nitrogen starvation for 24 hr in N_2-medium; this is because the mutant does not grow in N_2-medium. After incubation, the N_2- and glutamine-grown cells were washed, resuspended in HEPES buffer and used for [^{14}C]-glutamine uptake as described in Material and Methods. Values are means from two independent experiments, each with two replicates. N_2-medium: parent, (□); mutant, (■); glutamine-medium: parent (○); mutant, (●); glutamine-medium + chloramphenicol: parent, (▲)]

nmol/min/mg Chl *a*, and in the case of asparagine-grown Het⁻Nif⁻ mutant, the rates increased to 89 and 37 nmol/min/mg Chl *a*, during first and second phase, respectively (Fig. 2).

The N_2-grown parent strain showed arginine uptake rates of 34 and 21 nmol/min/mg Chl *a* and in N_2-medium, Het⁻Nif⁻ mutant took up arginine at rates of 60 and 31 nmol/min/mg Chl *a*, during first and second phase, respectively. The rates increased in the arginine-grown parent strain to 115 and 51 nmol/min/mg Chl *a*, and in mutant strain to 135 and 50 nmol/min/mg Chl *a*, during first and second phase, respectively (Fig. 3).

These results indicate that glutamine, asparagine and arginine uptake in *Nostoc* ANTH and its Het⁻Nif⁻ mutant are biphasic and substrate-inducible. The induction of amino acid uptake by the substrate required *de novo* protein synthesis since chloramphenicol prevented such induction. Unlike ammonium uptake, the uptake of glutamine, asparagine and arginine was not repressed by nitrate or ammonium, indicating that the uptake of such amino acids is not under strict nitrogen control, but

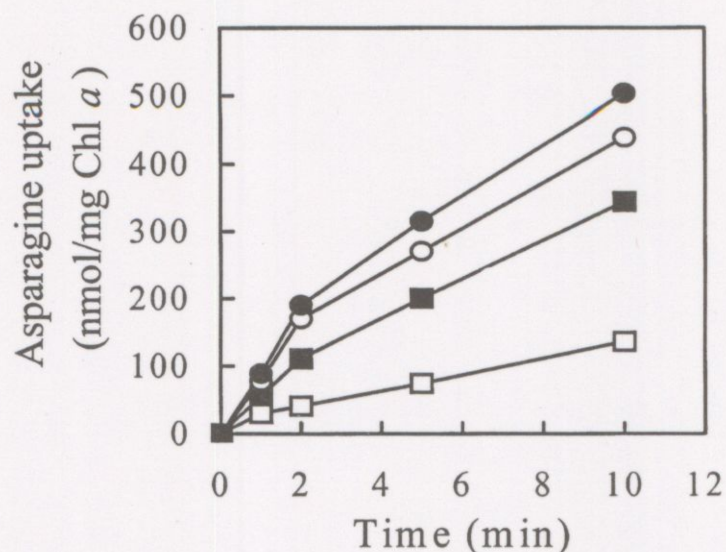

Fig. 2—[^{14}C]-Asparagine uptake in *Nostoc* ANTH and its Het⁻Nif⁻ mutant [Nitrate-grown exponential cultures were washed and transferred to N_2- ($BG11_0$) and asparagine- ($BG11_0$ + 1m*M* asparagine) media and incubated for 48 hr. The N_2-grown cells in case of mutant refer to the nitrate-grown cells, which were subjected to nitrogen starvation for 24 hr in N_2-medium; this is because the mutant does not grow in N_2-medium. Thereafter, the N_2- and asparagine-grown cells were washed, resuspended in HEPES buffer and used for [^{14}C]-asparagine uptake. Values are means from two independent experiments, each with two replicates. N_2-medium: parent, (□); mutant, (■); asparagine-medium: parent, (○); mutant, (●)]

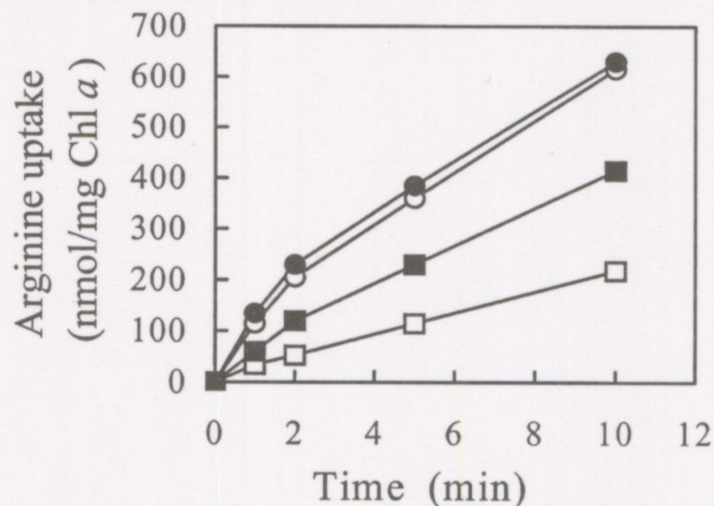

Fig. 3—[^{14}C]-Arginine uptake in *Nostoc* ANTH and its Het⁻Nif⁻ mutant [Nitrate-grown exponential cultures were washed and transferred to N_2- ($BG11_0$) and arginine- ($BG11_0$ + 1 m*M* arginine) media and incubated for 48 hr. Thereafter, the N_2- and arginine-grown cells were washed, resuspended in HEPES buffer and used for [^{14}C]-arginine uptake. The N_2-grown cells in case of mutant refer to the nitrate-grown cells, which were subjected to nitrogen starvation for 24 hr in N_2-medium; this is because the mutant does not grow in N_2-medium. Values are means from two independent experiments, each with two replicates. N_2-medium: parent, (□); mutant, (■); arginine-medium: parent, (○); mutant, (●)]

may be regulated by the nitrogen status of the cell. This is consistent with the lack of nitrate and ammonium inhibition of leucine uptake in *A. variabilis*[7] and arginine uptake in *Anabaena* PCC

Table 4—Effect of DCMU, DCCD and CCCP on glutamine, asparagine and arginine uptake by *Nostoc* ANTH

[Nitrate-grown exponential cultures were washed and transferred to glutamine-, asparagine- and arginine-media and incubated for 48 hr. The cells were then washed and resuspended in HEPES buffer. [^{14}C]-Glutamine, [^{14}C]-asparagine and [^{14}C]-arginine were added after 30 min of incubation in light with or without DCMU (10 μ*M*), DCCD (50 μ*M*) and CCCP (25 μ*M*). The uptake rates were calculated from the linear phase between 2 and 10 min after ^{14}C incorporation into the cells. Values presented are means ± SE from two independent experiments, each with two replicates]

Treatment	Uptake rates (nmol/mg Chl *a*/min)		
	Glutamine	Asparagine	Arginine
Control	44 ± 0.2	29 ± 0.5	51 ± 0.5
+DCMU	21 ± 1.0	15 ± 0.1	26 ± 0.8
+DCCD	4.1 ± 0.6	7.2 ± 1.3	9.1 ± 1.1
+CCCP	3.8 ± 0.4	4.0 ± 0.2	5.3 ± 0.1

7120[1]. In N_2-medium, the Het$^-$Nif$^-$ mutant showed a much higher rate of amino acid uptake than the parent because of nitrogen starvation caused by its inability to use N_2 as nitrogen source. However, both the mutant and the parent strains had similar uptake rates when grown in media containing the amino acids. A similar increase in rates of glutamine uptake was reported in the Het$^-$Nif$^-$ mutants of *A. variabilis* and this increase correlated well higher levels of glutamine in glutamine-grown cells compared to ammonium- or N_2-grown cells[6].

The effect of DCMU (an inhibitor of non-cyclic photosynthetic electron transport), DCCD (an inhibitor of F_0 part of ATPase) and CCCP (an uncoupler) was studied on the uptake rates of glutamine, asparagine and arginine in the parent strain (Table 4). The protonophore CCCP caused a strong inhibition of glutamine, asparagine and arginine uptake; a similar inhibition was also observed in the presence of the ATPase inhibitor, DCCD. The inhibitor of photosynthetic O_2-evolution, DCMU, partially inhibited the uptake of glutamine, asparagine and arginine. These inhibitors had a similar effect on uptake of amino acids by the Het$^-$Nif$^-$ mutant (data not shown). The results indicate that the cellular uptake of amino acids in *Nostoc* ANTH and its mutant is energy-dependent. Our results are in agreement with energy-dependent amino acid uptake in *Anabaena* PCC 7120 and *A. variabilis*[1,7,9].

To conclude, our results show that *Nostoc* ANTH and its Het$^-$Nif$^-$ mutant can grow at the expense of glutamine, asparagine and arginine as a source of fixed-nitrogen. Glutamine and asparagine repressed N_2-fixation and served as a sole source of fixed-nitrogen for growth. Arginine was a comparatively poorer source of nitrogen for growth and did not fully repress N_2-fixation or heterocyst formation. The poor growth in arginine-medium was not due to poor uptake rates since the uptake rates for arginine were not significantly different from those for glutamine or asparagine. The glutamine synthetase activity remained unaffected during cultivation in media containing any one of the three amino acids tested. The uptake of amino acids was substrate-inducible, energy-dependent, and required *de novo* protein synthesis. Nitrate and ammonium repressed ammonium uptake, but did not repress uptake of amino acids. In N_2-medium, the uptake of ammonium and amino acids in the Het$^-$Nif$^-$ mutant were significantly higher than that in its parent strain. It is suggested that the mutational loss of diazotrophy in the mutant resulted in nitrogen starvation when N_2 was the sole source of available nitrogen leading to highly derepressed ammonium and amino acid transport systems.

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