Effect of Glutamine on Growth and Heterocyst Differentiation in the Cyanobacterium Anabaena variabilis

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Mutants of the cyanobacterium Anabaena variabilis that were capable of increased uptake of glutamine, as compared with that in the parental strains, were isolated. Growth of these mutants and their parental strains was measured in media containing N₂, ammonia, or glutamine as a source of nitrogen. All strains grew well with any one of these sources of fixed nitrogen. Much of the glutamine taken up by the cells was converted to glutamate. The concentrations of glutamine, glutamate, arginine, ornithine, and citrulline in free amino acid pools in glutamine-grown cells were high compared with the concentrations of these amino acids in ammonia-grown or N₂-grown cells. All strains capable of heterocyst differentiation, including a strain which produced nonfunctional heterocysts, grew and formed heterocysts in the presence of glutamine. However, nitrogenase activity was repressed in glutamine-grown cells. Glutamine may not be the molecule directly responsible for repression of the differentiation of heterocysts.

Heterocysts, differentiated cells formed by many filamentous cyanobacteria, develop in response to deprivation of sources of fixed nitrogen. They are the sites of nitrogen fixation during aerobic growth (7, 9, 13, 18). Control of heterocyst differentiation and of the pattern that is formed by heterocysts in a filament is poorly understood. A source of fixed nitrogen, such as ammonia or nitrate, in the medium inhibits heterocyst formation in most Anabaena strains. Because heterocysts fix nitrogen and new heterocysts form about midway between existing heterocysts, a nitrogenous product of heterocyst metabolism may inhibit nearby cells in the filament from differentiating into heterocysts (7). It was at first thought that ammonia, the product of nitrogen fixation, might be the repressor of heterocyst differentiation (10); however, ammonia does not always repress heterocysts. Anabaena sp. strain CA naturally forms heterocysts in the presence of ammonia (2). Methionine-sulffoximine (MSX), which inhibits glutamine synthetase, allows heterocysts to form in the presence of ammonia (17, 28), as do other compounds, such as β-thiencylalanine (24) and some tryptophan analogs (3, 24). The mechanism(s) by which heterocyst differentiation is repressed during growth on a source of fixed nitrogen is not known; however, it has been suggested that glutamine or a product of the metabolism of glutamine, rather than ammonia, is the regulatory molecule (29). Several observations provide evidence that glutamine represses heterocyst development. (i) MSX inhibits the synthesis of glutamine (15, 28), but the addition of glutamine to cultures containing both MSX and ammonia results in the suppression of heterocysts (17). (ii) Glutamine is the first organic product of nitrogen fixation and is the nitrogenous molecule that is transported from heterocysts to vegetative cells (16, 29, 33). (iii) Anabaena azollae is a symbiotic cyanobacterium which excretes ammonia into the leaf of its water-fern host; under symbiotic conditions filaments of A. azollae may contain up to 30% heterocysts, but glutamine synthetase activity is 50% lower in A. azollae under these conditions than in A. cylindrica (22). The glutamine which is synthesized in the heterocysts moves to vegetative cells, where it is converted to glutamate by glutamate synthase, an enzyme that is found primarily (11), if not exclusively (19, 29), in vegetative cells.

Although auxotrophic mutants of Anabaena variabilis have been isolated (6), none requires glutamine. An MSX-resistant mutant strain of A. variabilis that requires glutamine, but only when grown on N₂, NO₃, or low concentrations of ammonia, has recently been reported (26). It has been shown that glutamine is taken up by filaments of A. variabilis but growth of the cells on glutamine as the sole nitrogen source occurred only at high concentrations of the amino acid; this growth, which occurred under an Ar-Ο₂-CO₂ atmosphere, was attributed to ammonia produced from glutamine (5). It has been shown that glutamine represses heterocysts; however, it was suggested that the repression could be attributed to ammonia produced by the breakdown of glutamine (30). More recently, it has been reported that Anabaena sp. strain CA can grow in a medium containing 5 mM glutamine and that growth on glutamine represses nitrogenase (21). The effect of glutamine on heterocysts was not reported. From two mutant strains of A. variabilis that are Nif⁻ (one, NF12, is capable of producing heterocysts and the other, NF76, is not) (6), we have isolated strains which grow well with glutamine as the sole source of fixed nitrogen. We report here the growth of A. variabilis on glutamine as the sole source of fixed nitrogen and describe the effect of glutamine on heterocyst differentiation, nitrogenase activity, and amino acid composition of filaments.

MATERIALS AND METHODS

Strains. Axenic cultures of A. variabilis Kütz. (ATCC 29413) and mutants of this strain (NF12 and NF76) which were previously isolated by nitrosoguanidine mutagenesis and penicillin enrichment (6, 12) were obtained from C. P. Wolk (MSU-DOE Plant Research Laboratory, Michigan State University, East Lansing). The isolation of mutants GNT1 and GNT2 is described below. The relevant characteristics of all of these strains are shown in Table 1.

Growth. Cultures were grown in 50-ml volumes in 125-ml flasks in medium BG-11 (23) or in an eightfold dilution of the medium of Allen and Arnon (AA/8) (1). The AA/8 medium was supplemented in some experiments either with 5 mM...
NH$_4$Cl and 10 mM N-tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid (TES) (pH 7.2) or with 1, 5, or 10 mM glutamine and 10 mM TES (pH 7.2). The solid medium (AA agar) was that of Allen and Arnon (1) supplemented in some experiments with ammonia or glutamine and solidified with agar (10 g liter$^{-1}$) (Difco Laboratories, Detroit, Mich.) purified by the method of Braun and Wood (4). Cultures were incubated at 30°C on a reciprocal shaker at 100 rpm under cool-white fluorescent lamps at an intensity of approximately 60 microEinsteins m$^{-2}$ s$^{-1}$. Growth was measured as the turbidity at 700 nm in a 1.0-cm light path in a Spectronic 70 spectrophotometer (Bausch & Lomb, Inc.).

**Ammonia measurements.** Duplicate cultures of wild-type and mutant GNT1 strains were grown in AA/8-5 mM ammonia–10 mM TES (pH 7.2) to a concentration of about 2 μg of chlorophyll (Chl) a ml$^{-1}$. MSX was added to one culture of each strain at a final concentration of 500 μM. Cells were incubated for 16 h at 30°C in the light with shaking. Cells were washed three times with 50-ml volumes of AA/8 and suspended in AA/8-10 mM TES (pH 7.2). Ammonium chloride was added at a concentration of 200 μM to portions of MSX-treated and untreated cells. Glutamine was added at a final concentration of 40 mM to a portion of MSX-treated cells. Cells were incubated as described above. Immediately after and at 2, 4, 6, 12, and 24 h after the addition of ammonium chloride or glutamine, 1.0-ml samples were removed. Cells were removed by centrifugation, and a portion of the supernatant solution was assayed for ammonia by a glutamate dehydrogenase assay (8) with ammonium chloride serving as the standard.

**Mutagenesis.** Cultures of strains NF12 and NF76 were grown in AA/8-ammonia, washed, and suspended in AA/8 at 4 x 10$^6$ cells ml$^{-1}$. Cells (5 ml) in a 100-mm petri dish were exposed with constant stirring to 10,000 ergs of UV light cm$^{-2}$ from a Sylvania germicidal (G30T8) lamp. There was 99 to 99.9% killing of cells under these conditions. Mutagenized cells were kept in the dark for 24 h, washed three times in AA/8, and spread on 10 plates of AA agar containing 5 mM glutamine and 10 mM TES (pH 7.2). Colonies first appeared after about 1 week and were immediately restreaked on the same medium. Isolated colonies from the second plate were grown in liquid medium (AA/8-5 mM glutamine–10 mM TES (pH 7.2)) and then tested for their ability to grow with glutamine as the sole nitrogen source and for their continued inability to grow under aerobic conditions without a source of oxidized nitrogen.

**Glutamine uptake.** Five milliliters of a culture grown in medium BG-11 to an optical density at 700 nm of approximately 0.3 was placed in a 125-ml flask and incubated at 32°C with shaking under cool-white fluorescent lights. [14C]Glutamine (267 Ci/mol; New England Nuclear Corp., Boston, Mass.) at a final concentration of 0.1 μCi ml$^{-1}$ (0.375 μM) was added. Duplicate 0.5-ml samples were removed and immediately added to 10 ml of ice-cold AA/8 containing 10 mM glutamine. Cells were washed by centrifugation five times with 10 ml of ice-cold AA/8-10 mM glutamine, and their radioactivity was measured in 5 ml of Aquasol scintillation fluid (New England Nuclear).

**Pulse-chase experiments.** Pulse-chase experiments were performed as follows. Strain GNT1 grown in AA/8-5 mM glutamine–10 mM TES (pH 7.2) was washed in AA/8, suspended in 15 ml of AA/8-10 mM TES containing 0.5 μCi of [14C]glutamine ml$^{-1}$ (1.9 μM), and incubated in the light with shaking at 32°C. After 5 min, duplicate 1.0-ml samples were transferred to tubes containing 9.0 ml of ice-cold AA/8-10 mM glutamine. Samples were similarly transferred after 15 min, and the remainder of the culture was quickly washed and suspended in AA/8-5 mM glutamine–10 mM TES to yield the same cell density as before washing. At 0.5, 1, and 2 h after the initiation of the experiment, duplicate 1.0-ml samples were removed to cold AA/8-10 mM glutamine. Samples were washed four times with AA/8 and suspended in 0.4 ml of 1.0 mM Tris (pH 8)-1.0 mM EDTA. Lysozyme was added to a final concentration of 0.5 mg ml$^{-1}$ and, after incubation for 30 min at room temperature, cells were frozen and thawed three to four times until lysis was complete. Lipids were extracted with a biphasic mixture of CHCl$_3$, methanol, and water (2:4.1:0.1:9 [vol/vol]), the aqueous phase of which was then reextracted with CHCl$_3$. The lipid-free, methanol-water phase was centrifuged at 12,000 × g for 30 min to precipitate protein. The supernatant solution was combined with an 80% methanolic extract of the pellet. The combined extracts were evaporated to dryness, suspended in 10 μl of 80% methanol, and applied to Silica Gel G thin-layer chromatography plates with standards containing unlabeled glutamine and glutamate. The plates were developed in butanol-acetic acid-water (80:20:20 [vol/vol])). Glutamine and glutamate were visualized with ninhydrin. The areas corresponding to these amino acids and the remaining material in each lane were recovered, and the radioactivity was measured in Aquasol scintillation fluid.

**Pools of amino acids.** The wild-type and mutant GNT1 strains were grown for 5 days in AA/8-10 mM TES (pH 7.2) with 5 mM ammonia or 5 mM glutamine or without a source of fixed nitrogen. Cells were washed by filtration five times with 50-ml volumes of AA/8 and concentrated to about 300 μg of Chl a ml$^{-1}$. Amino acids were extracted with 80% methanol either directly from 0.1 ml of whole cells or from 0.1 ml of cells which had been lysed by sonication (model 200 Sonifier; Branson Sonic Power Co., Danbury, Conn.) in tubes held in an ice-salt bath. No significant differences in amino acid pools were seen for the two methods of extraction. (The data presented in Table 3 are from experiments in which cells were extracted by the latter method.)

### TABLE 1. Characteristics of A. variabilis strains

<table>
<thead>
<tr>
<th>Strain</th>
<th>Parent</th>
<th>Phenotype</th>
<th>Rate of glutamine uptake$^c$</th>
<th>Source or reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild type</td>
<td>Wild type</td>
<td>+ Normal</td>
<td>31.5 ± 1.1</td>
<td>6</td>
</tr>
<tr>
<td>NF12</td>
<td>Wild type</td>
<td>+ Increased</td>
<td>14.8 ± 1.2</td>
<td>6</td>
</tr>
<tr>
<td>GNT1</td>
<td>NF12</td>
<td>+ Increased</td>
<td>91.4 ± 0.5</td>
<td>This study</td>
</tr>
<tr>
<td>NF76</td>
<td>Wild type</td>
<td>- None</td>
<td>45.5 ± 0.6</td>
<td>6</td>
</tr>
<tr>
<td>GNT2</td>
<td>NF76</td>
<td>- None</td>
<td>57.1 ± 0.9</td>
<td>This study</td>
</tr>
</tbody>
</table>

$^a$ Ability to grow on N$_2$ as the sole nitrogen source.

$^b$ Relative number of heterocysts during growth on N$_2$ as compared with that in the wild-type strain.

$^c$ Picomoles of [14C]glutamine incorporated minute$^{-1}$ milligrams of Chl a$^{-1}$; average of three to four determinations ± standard deviation.
lipid and protein were removed from the extracts as described for the sample preparation for thin-layer chromatography in the pulse-chase experiments. Residual lipid and protein were removed by passing the samples through a Set-pak column (Waters Associates, Inc., Milford, Mass.). Amino acids were measured in an amino acid analyzer (Beckman Instruments, Inc., Fullerton, Calif.) with a lithium-based buffer system for physiological fluids. A portion of the cell suspension was used for the quantitation of Chl a (14).

Heterocyst frequency. The ratio of mature heterocysts with clearly visible refractile polar regions to total cells was determined by counting 1,000 to 2,000 cells by light microscopy at a magnification of 600x.

Nitrogenase. Nitrogenase was assayed by acetylene reduction (27). Pure acetylene gas (3 ml) was added to each of two serum-stoppered 50-ml flasks containing 17 ml of cells. Flasks were incubated for 1 h at 30°C with agitation in the light. Duplicate samples were removed from each flask, and ethylene was measured in a Varian gas chromatograph with an 80-cm stainless steel column filled with Poropak R (80/100 mesh).

RESULTS

Glutamine transport mutants. We screened approximately 10^9 mutagenized cells of A. variabilis NF12 and NF76 for mutants that grew more rapidly than the parental strains on agar medium containing 5 mM glutamine as the sole source of fixed nitrogen. Of about 100 strains that we identified, only 2 that grew well on glutamine plates failed to grow on a medium lacking any source of fixed nitrogen. The mutant of strain NF12 that had enhanced growth on glutamine was called GNT1, and the analogous mutant of strain NF76 was called GNT2. Although neither strain appeared to grow without a source of fixed nitrogen in the initial screening, we subsequently found that GNT1 did grow in liquid medium with a 771

without fixed nitrogen; hence, it has a Nif^- phenotype. This strain was not a wild-type revertant of strain NF12, since it retained the characteristic of a high frequency of heterocysts of the NF12 parental strain and had lower nitrogenase activity than did the wild-type strain (see results below). The phenotypes of all strains used in this study and the rate at which they took up glutamine are shown in Table 1.

Growth. We compared the growth of all five strains in media either without a source of fixed nitrogen or containing either ammonia or glutamine as the sole source of fixed nitrogen. Strains NF12, NF76, and GNT2 are Nif^- and did not grow on N_2 as the sole nitrogen source; wild-type A. variabilis and strain GNT1 grew equally well with or without a source of fixed nitrogen (data not shown). All five strains grew well on ammonia or on glutamine at 1, 5, or 10 mM (data not shown). Strain GNT2 grew better than its parental strain, NF76, at all concentrations of glutamine (data not shown). Since strains NF12, NF76, and GNT2 used glutamine as the sole nitrogen source, it appeared that glutamine could support the growth of A. variabilis. Although these experiments did not prove that wild-type A. variabilis or strain GNT1 used glutamine as a source of nitrogen (since they can fix N_2), the evidence presented below supports the conclusion that they used glutamine for growth.

Ammonia determinations. Because the growth of Anabaena spp. on glutamine has previously been attributed to the breakdown of glutamine to ammonia (5, 30), either spontaneously or by a glutaminase in the cells, we thought it was important to measure the concentration of ammonia in cultures containing glutamine. Ammonia resulting from the breakdown of glutamine would be rapidly assimilated by growing cells; however, MSX-treated cells excrete ammonia into the culture medium (20). Therefore, cultures of the wild-type strain and mutant GNT1 were treated with MSX to prevent the assimilation of ammonia and to allow its excretion into the culture medium. We detected no accumulation

FIG. 1. Production of ammonia from glutamine. Filaments of the wild-type strain of A. variabilis (a concentration of 2 μg of Chl a ml^-1) were treated with MSX (500 μM) and then incubated (after being washed) with 40 mM glutamine (△) or 200 μM ammonium chloride (■). Filaments not treated with MSX were also incubated with 200 μM ammonium chloride (○).

FIG. 2. Pulse-chase experiment. Filaments of strain GNT1 were labeled with [14C]glutamine for 15 min, washed free of labeled glutamine, and grown for a further 105 min in a medium containing 5 mM glutamine. 14C-labeled glutamine (○), glutamate (■), and other compounds soluble in 80% methanol (△) were extracted from samples taken before and after the chase. The arrow indicates the beginning of the chase with unlabeled glutamine.
of ammonia in a culture medium containing glutamine even after 24 h of incubation with MSX-treated cells of the wild-type strain (Fig. 1). MSX-treated cells incubated in the presence of ammonia did not assimilate that ammonia, while the same cells not treated with MSX rapidly depleted ammonia from the culture medium (Fig. 1). Similar results were obtained for strains GNT1 and NF12 (data not shown). We detected 0.3 to 0.5% ammonia in aqueous solutions of glutamine, and this amount did not increase significantly over a period of several days, even if the solutions were left at room temperature. The ammonia in 5 mM glutamine could support the growth of non-nitrogen-fixing strains for only a few hours, and there was no evidence that significant amounts of ammonia were produced from glutamine. We conclude that the growth of strains NF12, NF76, and GNT1 on glutamine is not attributable to the breakdown of glutamine to ammonia.

**Pulse-chase experiments.** The distribution of $^{14}$C in glutamine, glutamate, and other 80% methanol-soluble components extracted from cells of strain GNT1 after 5 and 15 min of incorporation of $[^{14}]$C glutamine and after a chase with nonradioactively labeled glutamine is shown in Fig. 2. Glutamine was quickly converted to glutamate; however, even 105 min after the removal of $[^{14}]$C glutamine from the medium almost 50% of the methanol-soluble counts were still found in glutamine. Thus, the addition of exogenous glutamine increased the intracellular concentration of both glutamine and glutamate (see below).

**Pools of amino acids.** The levels of glutamine, glutamate, arginine, citrulline, and ornithine in cells of the wild-type and GNT1 strains grown with or without glutamine are shown in Table 2. Glutamine was not detected in either ammonia-grown cells or cells grown on $N_2$; however, there was a large pool of glutamine in cells grown on glutamine. Strain GNT1, which takes up glutamine better than the wild-type strain, had about four times as much glutamine as the wild-type strain. The glutamine-grown cells also had large amounts of glutamate, as would be expected from the high rate of conversion of glutamine to glutamate. Other amino acids in the glutamate family, citrulline, ornithine, and arginine, were also present in relatively large amounts in glutamine-grown cells. There were no significant differences in other amino acid pools between glutamine-grown and ammonia-grown or $N_2$-grown cells (data not shown).

**Heterocysts.** The frequency of heterocysts was measured after 24, 48, and 72 h of growth of the wild-type, NF12, and GNT1 strains on $N_2$, ammonia, or glutamine (Table 3). Strains NF76 and GNT2 did not produce any heterocysts in any of these media. In general, strain GNT1 produced more heterocysts than did the other two strains. In addition, maximum heterocyst production for strain GNT1 grown on $N_2$ occurred at 24 h; heterocyst production was slightly inhibited by glutamine. The other two strains produced the most heterocysts at 48 h. Although glutamine slightly inhibited the frequency of heterocysts at 48 h in strain NF12, there was no such inhibition in the wild-type strain. Heterocyst frequencies remained fairly constant for all the strains beyond 72 h (data not shown). The most significant result is that strain NF12, which cannot fix nitrogen, produced heterocysts as if it were starved for fixed nitrogen when it was grown on glutamine.

**Acetylene reduction.** We measured acetylene reduction by the heterocyst-producing strains grown with $N_2$ and either with or without $5 \text{mM}$ glutamine for 48 h prior to the assay. As expected, strain NF12 did not reduce acetylene under either condition of growth (Table 4). Both the wild-type and

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**TABLE 2. Pools of amino acids of A. variabilis**

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Wild type grown on:</th>
<th>GNT1 grown on:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$N_2$</td>
<td>$NH_3$</td>
</tr>
<tr>
<td>Glutamate</td>
<td>0.33</td>
<td>0.50</td>
</tr>
<tr>
<td>Glutamine</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Arginine</td>
<td>&lt;0.01</td>
<td>0.27</td>
</tr>
<tr>
<td>Citrulline</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Ornithine</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

*a* Determined with an amino acid analyzer.

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**TABLE 3. Heterocyst frequencies in A. variabilis**

<table>
<thead>
<tr>
<th>Time (h) after N shift</th>
<th>Strain</th>
<th>% Heterocysts in strains grown on:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>NH$_4$Cl</td>
</tr>
<tr>
<td>24</td>
<td>Wild type</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td></td>
<td>NF12</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td></td>
<td>GNT1</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>48</td>
<td>Wild type</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td></td>
<td>NF12</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td></td>
<td>GNT1</td>
<td>0.7 ± 0.6</td>
</tr>
<tr>
<td>72</td>
<td>Wild type</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td></td>
<td>NF12</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td></td>
<td>GNT1</td>
<td>0.4 ± 0.3</td>
</tr>
</tbody>
</table>

*a* Average of three to four determinations ± standard deviation. Ammonia-grown cells were washed and then suspended and grown in media containing the nitrogen sources indicated.

$^b$ A single determination.
GNT1 strains reduced acetylene. Despite the ability of strain GNT1 to grow as well as the wild-type strain on N₂, it had reduced nitrogenase activity as compared with the wild-type strain (Table 4). Heterocysts of the wild-type strain reduced much less acetylene when grown in the presence of 5 mM glutamine than when grown on N₂ as the nitrogen source, and heterocysts of strain GNT1 grown on glutamine had virtually no nitrogenase activity. Therefore, in the presence of glutamine strain GNT1 must depend on that glutamine for growth.

### DISCUSSION

All strains of *A. variabilis* used in these experiments grew well on 1, 5, and 10 mM glutamine. It has been suggested that the growth of *Anabaena* spp. on glutamine may represent growth on ammonia which comes from the breakdown of glutamine to glutamate and ammonia (5, 30). We found that the concentration of ammonia in glutamine at concentrations as high as 40 mM was insufficient to support the growth of a non-nitrogen-fixing strain for more than a few hours. MSX-treated cells did not produce significant amounts of ammonia from glutamate; hence, it does not appear that large pools of intracellular ammonia derived from glutaminase activity could account for growth on glutamine. The most reasonable conclusion is that these *A. variabilis* strains use glutamine as a source of fixed nitrogen. Although the growth experiments alone proved that the Nif⁻ strains used glutamine as the sole nitrogen source, it could be argued that the Nif⁻ strains fixed N₂ and either did not take up or did not use glutamine. The large size of the pools of glutamate and glutamate in these two strains strongly suggests that glutamate is both taken up and metabolized by the Nif⁺ strains. In addition, nitrogenase activity was reduced by more than 60 and 98% in the wild-type and GNT1 strains, respectively, during growth on glutamine; the inhibition of nitrogenase by glutamine has also been reported for *Anabaena* sp. strain CA (21) and for *A. cylindrica* (25).

The maintenance of the pattern of heterocysts in a filament may be regulated by a gradient of some molecule(s) which is the product of nitrogen fixation (30, 32). Although ammonia was first suggested as the regulatory molecule (10), the fact that MSX allowed the heterocyst pattern to be maintained even in the presence of ammonia argued against this idea. It has been suggested that glutamine or some product of glutamine metabolism may regulate pattern formation (29). Wolk (31) has discussed in some detail models for an inhibitory field around heterocysts; it has been proposed that diffusion of an inhibitory substance that is metabolized could account for the pattern formation that is seen in *Anabaena* spp. (30, 32). If that inhibitor is glutamine, then the influx of large amounts of glutamine into all vegetative cells in the filament should suppress further heterocyst differentiation.

Based on the facts that (i) the heterocysts of the strains we examined do not fix N₂ (or fix it poorly) in the presence of glutamine and, therefore, are presumably not producing glutamine and (ii) the intracellular concentration of glutamine is high in glutamine-grown filaments, we suggest that there is no significant glutamine gradient in these filaments and that glutamine per se may not be the molecule that represses heterocyst differentiation.

### ACKNOWLEDGMENTS

We are grateful to C. P. Wolk for cyanobacterial strains and for valuable discussions. We thank Sheri Bonar for technical assistance, Gregory Grant and Michael Burg for performing the amino acid analyses, and Marcia Harrison for help in the acetylene reduction assays.

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### LITERATURE CITED


### TABLE 4. Nitrogenase activity

<table>
<thead>
<tr>
<th>N source for growth</th>
<th>Strain</th>
<th>Nitrogenase activity (μmol of ethylene produced minute⁻¹ mg of Chl a⁻¹)</th>
<th>% Heterocysts</th>
</tr>
</thead>
<tbody>
<tr>
<td>N₂</td>
<td>Wild type</td>
<td>27.55</td>
<td>7.4</td>
</tr>
<tr>
<td></td>
<td>NF12</td>
<td>&lt;0.1</td>
<td>10.0</td>
</tr>
<tr>
<td></td>
<td>GNT1</td>
<td>6.35</td>
<td>16.7</td>
</tr>
<tr>
<td>Glutamine</td>
<td>Wild type</td>
<td>7.42</td>
<td>5.3</td>
</tr>
<tr>
<td></td>
<td>NF12</td>
<td>&lt;0.1</td>
<td>9.5</td>
</tr>
<tr>
<td></td>
<td>GNT1</td>
<td>0.27</td>
<td>11.4</td>
</tr>
</tbody>
</table>

* Cells were grown for 48 h with either N₂ or 5 mM glutamine as the nitrogen source.


