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DL-7-Azatryptophan and Citrulline Metabolism in the Cyanobacterium *Anabaena* sp. Strain 1F

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An alternative route for the primary assimilation of ammonia proceeds via glutamine synthetase-carbamyl phosphate synthetase and its inherent glutaminase activity in *Anabaena* sp. strain 1F, a marine filamentous, heterocystous cyanobacterium. Evidence for the presence of this possible alternative route to glutamate was provided by the use of amino acid analogs as specific enzyme inhibitors, enzymological studies, and radioistopic labeling experiments. The amino acid pool patterns of continuous cultures of *Anabaena* sp. strain 1F were markedly influenced by the nitrogen source. A relatively high concentration of glutamate was maintained in the amino acid pools of all cultures irrespective of the nitrogen source, reflecting the central role of glutamate in nitrogen metabolism. The addition of 1.0 μM azaserine increased the intracellular pools of glutamate and glutamine. All attempts to detect any enzymatic activity for glutamate synthase by measuring the formation of L-[^{14}C]glutamate from 2-keto-[1-^{14}C]glutarate and glutamine failed. The addition of 10 μM DL-7-azatryptophan caused a transient accumulation of intracellular citrulline and alanine which was not affected by the presence of chloramphenicol. The in vitro activity of carbamyl phosphate synthetase and glutaminase increased severalfold in the presence of azatryptophan. Results from radioisotopic labeling experiments with [^{14}C]bicarbonate and L-[1-^{14}C]ornithine also indicated that citrulline was formed via carbamyl phosphate synthetase and ornithine transcarbamylase. In addition to its effects on nitrogen metabolism, azatryptophan also affected carbon metabolism by inhibiting photosynthetic carbon assimilation and photosynthetic oxygen evolution.

In the filamentous, heterocystous cyanobacteria *Anabaena* spp., as in most microorganisms, the assimilation of ammonia proceeds by the glutamine synthetase (EC 6.3.1.2)-glutamate synthase (GOGAT; EC 1.4.7.1) pathway. Evidence for the presence of this pathway has been provided by enzymological studies (6, 8, 14, 16, 19, 20), radioisotope tracer experiments (13, 26), and the use of amino acid analogs (3, 11, 21, 24).

Although the role of amino acid analogs in in vivo systems may be difficult to interpret since they may have multiple effects, amino acid analogs have been extensively used in many detailed studies to determine much about amino acid and protein synthesis, cell structure, and cell development. Moreover, amino acid analogs can be used as a tool, along with radioisotope tracer experiments and enzymological studies, to discern the path by which ammonia is assimilated in a variety of organisms.

Previously, it was reported that DL-7-azatryptophan (AZAT), a tryptophan analog, can initiate the formation of heterocysts from undifferentiated and normally repressed cultures of *Anabaena* sp. strain CA (3, 21). It also caused effects symptomatic of generalized nitrogen starvation. GOGAT is inhibited by AZAT, and there is an increase in C-phycocyanin proteolysis which correlates with the onset of heterocyst differentiation (5). However, when comparing the effect of AZAT on other *Anabaena* strains, we found that several strains did not respond to AZAT like *Anabaena* sp. strain CA (C. H. Chen, C. Van Baalen, and F. R. Tabita, Abstr. Annu. Meet. Am. Soc. Microbiol. 1984, K142, p. 170). In this investigation, we examine the relationship between nitrogen metabolism and heterocyst development in *Anabaena* sp. strain 1F and present evidence for the role of AZAT in mediating changes in primary ammonia assimilation.

MATERIALS AND METHODS

Organism. *Anabaena* sp. strain 1F and *Anabaena* sp. strain CA (ATCC 33047) are two rapidly growing marine filamentous, heterocystous cyanobacteria which have been described previously (7, 22). Doubling times are between 4.5 and 5.5 h in either nitrogen-free or combined nitrogen-supplemented basal salts medium.

Culture conditions. Strains 1F and CA were routinely grown in ASP-2 medium in batch or continuous cultures at 39°C. The growth conditions and continuous culture systems have been described in detail elsewhere (5).

Extraction and determination of intracellular amino acid pools. Intracellular amino acid pools of cultures of *Anabaena* sp. strain 1F were extracted with 70% (vol/vol) cold ethanol by the method of Dharmawardene and Stewart (6) with a slight modification. The intracellular amino acid pools were determined under the conditions described previously (2, 5).

Preparation of cell extracts. *Anabaena* sp. strain 1F was harvested by centrifugation at 5,000 × *g* for 5 min and washed twice with fresh ASP-2 medium. The cells were disrupted by two consecutive passages through a French pressure cell operated at 12,000 lb/in^2. The debris was removed by ultracentrifugation at 20,000 × *g* for 20 min. After dialysis against 10 mM imidazole buffer, pH 7.0, and 10 mM 2-mercaptoethanol overnight, the supernatant was used as the source of crude enzyme.

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Enzyme assay. GOGAT activity was determined by measuring the ferredoxin-dependent formation of L-[^{14}C]glutamate from 2-keto-[1-^{14}C]glutarate and unlabeled glutamine as described by Rai et al. (19). Glutaminase (EC 3.5.1.2) was assayed by measuring the conversion of L-[^{14}C]glutamine to L-[^{14}C]glutamate under conditions similar to those described by Prusiner and Milner (17). The reaction mixture (0.1 ml) contained 30 μmol of L-glutamine with 0.25 μCi of L-[^{14}C]glutamine (specific activity, 272 mCi/mmol) and 50 μmol of sodium acetate, pH 4.9. L-[^{14}C]glutamate formed from L-[^{14}C]glutamine was separated from the reaction mixture by the use of a Dowex-1 (Cl^-) ion exchange column (0.5 by 10 cm), and the radioactivity of L-[^{14}C]glutamate was counted on a Beckman liquid scintillation counter (model LS-100 C; Beckman Instrument Co., Palo Alto, Calif.). Carbamyl phosphate synthetase (EC 2.7.2.9) was determined by measuring dark, glutamine-dependent [^{14}C]bicarbonate fixation into carbamyl phosphate and coupling its formation to L-[^{14}C]citrulline formation in the presence of an excess of ornithine transcarbamylase. The crude enzyme extracts were assayed in a mixture containing 100 mM Tris chloride, pH 8.3, 10 mM glutamine, 10 mM sodium [^{14}C]bicarbonate (0.1 μCi/ml), 10 mM ATP, 10 mM $MgSO_4$, 10 mM L-ornithine, and 20 U of ornithine transcarbamylase. Unfixed [^{14}C]bicarbonate was acidified and driven off with 0.2 ml of 50% acetic acid. The radioactivity of the acidified sample was then quantified by liquid scintillation counting.

Protein determination. Whole-cell protein of *Anabaena* sp. strains 1F and CA was calculated from standard curves relating cell protein and culture turbidity. An A_{660} of 1.0 in a Spectronic 20 spectrophotometer with a 2.5-cm light path is equal to 170 μg of protein of strain 1F and 220 μg of protein of strain CA per ml. The protein content in cell extracts of *Anabaena* sp. strain 1F was determined by the Coomassie blue-binding method (4), with bovine serum albumin as the standard.

Pulse-labeling procedures. Long-term [^{14}C]bicarbonate and L-[1-^{14}C]ornithine pulse-labeling experiments were performed with 100-ml ASP-2 and nitrate-grown cultures of *Anabaena* sp. strain 1F (cell density, 200 μg [dry weight] per ml of culture) at 39°C. Radioactive compound (10 μCi per 100 ml of culture) was added to the cultures in the presence or absence of 10 μM AZAT. At various points, 10-ml samples were taken and washed twice with ASP-2 medium to remove residual radioactivity. After centrifugation at 5,000 × *g* for 5 min, 5 ml of cold 70% ethanol (vol/vol) was added to extract the intracellular amino acids. The ethanol extracts were concentrated with a freeze dryer (Virtis Co., Inc., Gardiner, N.Y.), and 1 ml of the appropriate buffer was then added. The ethanol extracts were used for radioactivity determination, chromatographic analysis, and identification of the ^{14}C-labeled compounds.

Chromatographic analysis and radioautography. Samples (10 μl) of the ethanol extracts were applied to the origin position of sheets of Polygram silica gel (20 by 20 cm; Brinkman Instruments, Inc., Westbury, N.Y.). The sheets of silica gel were equilibrated and analyzed by ascending chromatography in a butanol-acetic acid-water (4:1:1, by volume) solvent system. Radioautograms of the resulting thin-layer chromatograms were made with Kodak X-omat AR safety film for 7 days at −20°C to locate the positions of the labeled compounds.

Identification of labeled compounds. Amino acids were detected on thin-layer chromatograms by spraying with ninhydrin solution. Cochromatography with authentic radioisotopic amino acids served to identify labeled amino acids after radioautography. In addition, ninhydrin sprays were used to identify amino acids chromatographed with authentic standards.

Determination of photosynthetic activity. The photosynthetic activity of *Anabaena* sp. strains 1F and CA was measured by oxygen evolution with a YSI Clark-type oxygen electrode (no. 5331; Yellow Spring Instrument Co., Yellow Springs, Ohio) mounted in a water-jacketed cell at 35°C; 1.9 ml of the growing cultures, at an optical density of 0.5, was added to the chamber with a syringe. Changes in electrode current were detected and amplified with a microvolt ammeter. Illumination was provided by a saturating beam derived from a slide projector with a 300-W Sylvania light bulb, with a 10-cm water screen as the heat filter. The photosynthetic activity of *Anabaena* sp. strains 1F and CA was also determined by following the rate of [^{14}C]bicarbonate incorporation. A 5-ml sample was washed twice in fresh ASP-2 medium and placed in a vial flushed with dinitrogen gas, and then 20 mM sodium bicarbonate containing 1.0 μCi of [^{14}C]bicarbonate was added to each vial. Vials were placed in an illuminated Warburg apparatus with continuous shaking at 39°C. At various times, 0.5-ml samples were removed and immediately acidified with 0.2 ml of propionic acid to exclude unfixed bicarbonate. A tissue solubilizer (0.2 ml of Soluene 350; Packard Instrument Co. Inc., Rockville, Md.) was then added to each vial. Radioactivity was counted with a Beckman LS-100 C scintillation counter after the addition of 3 ml of Insta-Gel scintillation cocktail.

Chemicals. AZAT was purchased from the Sigma Chemical Co., St. Louis, Mo. Azaserine (AZAS) was obtained from Calbiochem-Behring Co., La Jolla, Calif. Radioisotopes of 2-keto-[1-^{14}C]glutaric acid, sodium salt (54 mCi/mmol) and L-U-[^{14}C]glutamine (272 mCi/mmol) were obtained from New England Nuclear Corp., Boston, Mass. Sodium [^{14}C]bicarbonate (20 mCi/mmol) and L-[*carbamoyl*-^{14}C]citrulline (55 mCi/mmol) were products of Amersham Co., Arlington Heights, Ill. L-[1-^{14}C]ornithine (50 mCi/mmol) was supplied by ICN Co., Irvine, Calif. All other chemicals were reagent grade.

RESULTS

Steady-state amino acid pools of ammonium- and nitrate-grown and dinitrogen-fixing cultures. The intracellular pools of amino acids found in steady-state cultures of *Anabaena* sp. strain 1F grown on 10 mM ammonium, nitrate, or dinitrogen were determined (Table 1). The steady-state amino acid pool patterns differed depending on the nitrogen source used for growth; the level of glutamate was significantly higher than that of other amino acids, particularly in dinitrogen- and nitrate-grown cells. Low to undetectable levels of glutamine were present in the dinitrogen-fixing and nitrate-grown cultures of *Anabaena* sp. strain 1F, although glutamine is the primary product of ammonia assimilation in *Anabaena* spp. (13, 26).

Changes in amino acid pools after addition of AZAT or AZAS. The addition of 1.0 μM AZAS, a glutamine analog which inhibits GOGAT activity and subsequently reduces the rate of conversion of glutamine to glutamate, caused a transient increase of the glutamine pool of nitrate-grown cultures of *Anabaena* sp. strain 1F. However, AZAS did not cause a decline in the glutamate pool (Table 2). These results suggested that a route(s) other than GOGAT might be used by *Anabaena* sp. strain 1F to regenerate glutamate from glutamine. The addition of AZAT to continuous cultures of *Anabaena* strain sp. 1F also led to a significant change in the

TABLE 1. Nitrogen source and steady-state amino acid pool patterns of *Anabaena* sp. strain 1F[a]

Amino acid	Amount (nmol/mg of protein)[b] in cells grown with:		
	Dinitrogen	Nitrate	Ammonia
Aspartic acid	1.807	1.376	4.178
Threonine	0.852	1.126	1.189
Serine	1.110	2.260	2.060
Glutamic acid	18.116	18.182	5.245
Glutamine	0.334	ND[c]	4.714
Glycine	0.666	1.130	1.154
Alanine	2.146	3.884	3.784
Cystine	5.540	5.428	2.907
Leucine	0.256	0.350	0.258
Ornithine	1.218	0.350	3.520

[a] Dinitrogen-fixing and nitrate- and ammonia-grown cells of *Anabaena* sp. strain 1F were grown under continuous culture conditions; 10 mM nitrate or ammonia was provided for nitrate- and ammonia-grown cultures, respectively. No asparagine, citrulline, valine, methionine, isoleucine, tyrosine, tryptophan, lysine, histidine, or arginine was detected.
[b] All values are the means of two separate determinations.
[c] ND, None detected.

pool patterns of amino acids over an 8-h period (Fig. 1). The intracellular pools of citrulline and alanine increased markedly. There were some fluctuations in the intracellular pools of glutamine and glutamate, but these changes were not nearly as pronounced as those in the citrulline and alanine levels. Since an amino acid is tentatively identified by its retention time on the amino acid analyzer column, it cannot be excluded that another ninhydrin-positive substance might coelute with citrulline, 1 of 42 ninhydrin-positive substances contained in the amino acid solution used to calibrate the amino acid analyzer (Sigma A 9906; Sigma Chemical Co.).

Effect of AZAT on distribution of ^{14}C-labeled constituents of the ethanol-soluble fraction. The labeled products of [^{14}C]bicarbonate or L-[1-^{14}C]ornithine incorporation by *Anabaena* sp. strain 1F treated with AZAT were analyzed by thin-layer chromatography and radioautography. Analyses showed that ^{14}C from ornithine or bicarbonate rapidly appeared in the intracellular amino acid pools (Fig. 2 and 3). In the presence of AZAT, one spot with an R_f identical to that of authentic citrulline was found and had the highest

TABLE 2. Effect of 1.0 μM AZAS on amino acid pools of nitrate-grown *Anabaena* sp. strain 1F[a]

Amino acid	Amount (nmol/mg of protein)[b] after exposure to AZAS for:				
	0 h	2 h	4 h	6 h	8 h
Aspartic acid	1.435	2.223	1.914	1.885	1.844
Threonine	1.476	2.065	2.230	2.230	3.258
Serine	2.245	3.443	2.439	2.679	3.141
Glutamic acid	17.883	19.568	19.889	19.811	18.775
Glutamine	—[c]	2.133	2.368	1.198	0.974
Glycine	1.519	1.393	1.792	2.274	2.260
Alanine	3.930	6.053	6.609	6.278	8.260
Cystine	3.555	5.523	3.998	4.456	4.812
Methionine	0.039	0.039	0.083	0.063	0.083
Isoleucine	0.151	0.136	0.224	0.253	0.128
Leucine	0.362	0.502	0.677	0.425	0.315
Ornithine	1.471	2.859	2.167	2.035	1.897

[a] Nitrate-grown *Anabaena* sp. strain 1F was grown in continuous culture to a cell density of 142 mg of protein per liter. No asparagine, citrulline, valine, tyrosine, tryptophan, lysine, histidine, or arginine was detected.
[b] All values are the means of two separate determinations.
[c] —, Not detected.

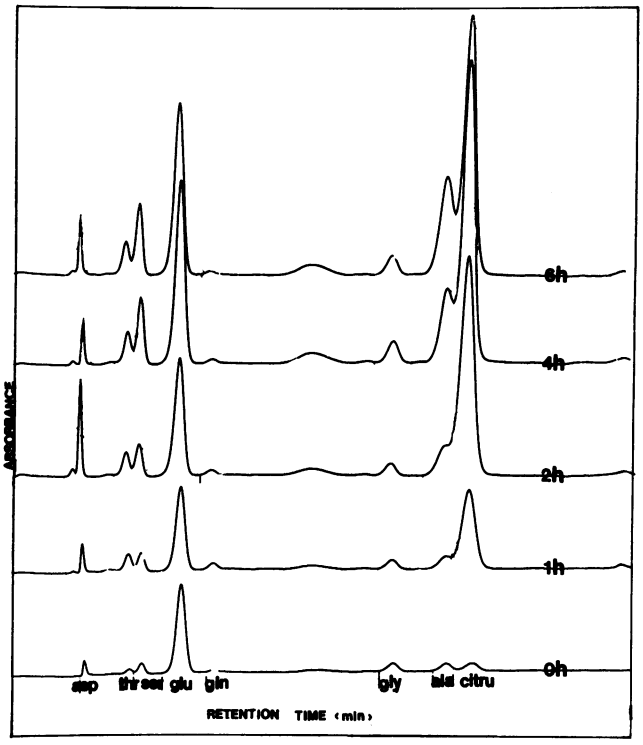

FIG. 1. Amino acid pool patterns of a nitrate-grown continuous culture of *Anabaena* sp. strain 1F treated with AZAT.

amount of radioactivity (accounting for more than 20% of the total radioactivity in the 70% ethanol extracts) among the radioactive spots. On the basis of these results, it appeared that citrulline, which accumulated in the presence of AZAT, became labeled when cells were allowed to incorporate [^{14}C]bicarbonate or L-[1-^{14}C]ornithine via the carbamyl phosphate synthetase-ornithine transcarbamylase pathway. Presumably, this occurs through the formation of carbamyl phosphate from glutamine or from ammonia, carbon dioxide, and ATP, followed by the condensation of carbamyl phosphate with ornithine to yield citrulline.

GOGAT, carbamyl phosphate synthetase, and glutaminase in *Anabaena* sp. strain 1F. In a reaction mixture (containing all constituents of the GOGAT assay: 2-keto-[1-^{14}C]glutarate, glutamine, aminooxyacetate, ferredoxin, and dithionite), neither toluene-permeabilized cells nor cell extracts of *Anabaena* sp. strain 1F showed detectable GOGAT activity. However, carbamyl phosphate synthetase activity increased 1.9-fold over a 5-h period, from a specific activity of 5.2 to a specific activity of 9.8 nmol of $H^{14}CO_3^-$ fixed per min per mg of protein. The specific activity of glutaminase increased 2.7-fold, from 3.2 to 8.7 nmol of [^{14}C]glutamate formed per min per mg of protein, over the same 5-h period. The increase in the activity of the two enzymes and the increase in the intracellular levels of citrulline (Fig. 1 to 3) were not affected by the presence of chloramphenicol, suggesting that protein synthesis may not be required (results not shown).

Effect of AZAT on photosynthetic activity. Photosynthetic activity was determined either by the rate of oxygen evolution or by the rate of [^{14}C]bicarbonate incorporation. Oxygen evolution activity declined dramatically over a 5-h period when nitrate-grown cultures of *Anabaena* sp. strain 1F or CA was treated with AZAT (Fig. 4). Although the addition of AZAT caused a temporary increase in the rate of bicarbon-

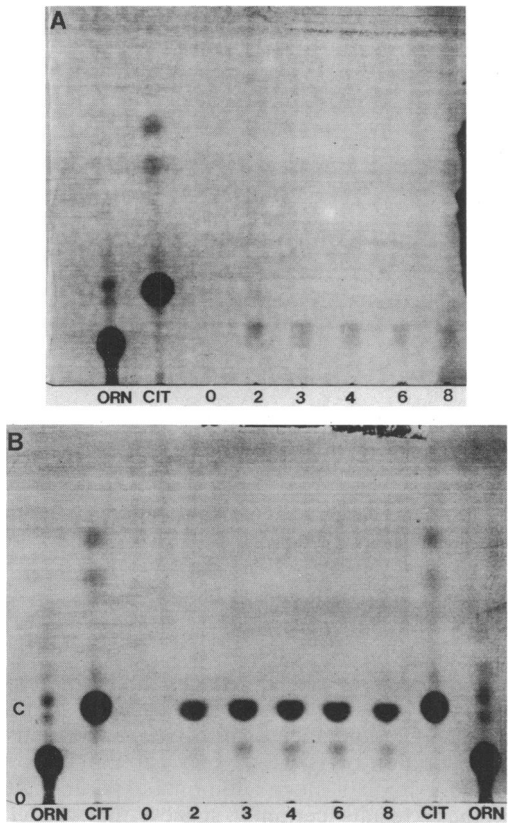

FIG. 2. Thin-layer chromatography of the products of L-[^{14}C]ornithine incorporation into *Anabaena* sp. strain 1F. Nitrate-grown cells were taken from continuous cultures in the absence (A) or presence (B) of 10 μM AZAT. At 0, 2, 3, 4, 6, and 8 h after the addition of AZAT, a 10-ml sample was removed from the culture and 70% ethanol extracts were prepared as described in Materials and Methods. O, Origin; C, L-[^{14}C]ornithine-labeled compound; ORN, standard L-[^{14}C]ornithine; CIT, standard L-[^{14}C]citrulline.

ate incorporation with *Anabaena* sp. strain 1F, eventually the rate of [^{14}C]bicarbonate incorporation was inhibited by AZAT, as was the case with *Anabaena* sp. strain CA (Fig. 5).

DISCUSSION

Glutamine synthetase and GOGAT are required for primary ammonia assimilation in *Anabaena* sp. strain CA (3, 5, 21). We have also found that development of heterocysts from undifferentiated filaments of this organism, inhibits the activity of glutamate synthase (5). However, unlike with *Anabaena* sp. strain CA, AZAT did not initiate the derepression of heterocyst synthesis in *Anabaena* sp. strain 1F, another rapidly growing marine filamentous, heterocystous cyanobacterium (C. H. Chen, C. Van Baalen, and F. R. Tabita, Abstr. Annu. Meet. Am. Soc. Microbiol. 1984).

AZAS (*O*-diazoacetyl-L-serine) is an inhibitor of a wide range of glutamine-dependent amino transferases (6) which shows a mixed type of inhibition with glutamine-requiring enzymes. Because AZAS and glutamine are structurally similar, they compete for binding to the active site; however, once bound, the diazoacetyl group of AZAS becomes irreversibly attached and cannot be competed with. Because of its reasonably specific nature, AZAS is quite useful in determining the pathway of ammonia assimilation. If the route from ammonia to amino nitrogen is via the amide of glutamine, then the addition of AZAS should block the production of glutamate and amino nitrogen and lead to the accumulation of glutamine. The addition of AZAS to a continuous culture of *Anabaena* sp. strain 1F indeed caused a transient accumulation of intracellular glutamine. However, the level of intracellular glutamate unexpectedly increased somewhat (Table 2). We have been unable, using a variety of enzyme preparations, to demonstrate the presence of GOGAT activity in this organism. Mixing experiments with active extracts of *Anabaena* sp. strain CA did not indicate that there was any obvious inhibitor in *Anabaena* sp. strain 1F extracts. We were also unable to show any effect of AZAS and AZAT on heterocyst development in this organism. However, the apparent absence of GOGAT activity may be because this enzyme is unstable in *Anabaena* sp. strain 1F or possesses some unusual requirement. Further experiments will be required to determine this. Early studies by Haystead et al. (9) reported the presence of glutaminase in cyanobacterial extracts, and Lea and Norris (11) reported that extracts of *Anabaena cylindrica* and *Nostoc ellipsosporium* were responsible for a significant amount of glutamate production from glutamine in the presence of aminooxyacetate, even when α-ketoglutarate, ferredoxin, and dithionite were omitted from the reaction mixture. Therefore, it is certainly not without precedent that alternative means to assimilate ammonia, in addition to the glutamine synthetase-GOGAT pathway, may be present in *Anabaena* sp. strain 1F in order to regenerate glutamate from glutamine in the presence of AZAS.

The finding that AZAT caused a transient accumulation of intracellular citrulline (Fig. 1) and an activation of carbamyl phosphate synthetase and glutaminase activity in *Anabaena* sp. strain 1F (which was not inhibited by chloramphenicol) seems to indicate that the carbamyl phosphate synthetase-glutaminase-ornithine transcarbamylase route is activated in this organism. Pulse-labeling studies with ^{14}C-labeled amino acids can usually delineate the main characteristics of a metabolic pathway (18), although there are some limitations to this technique for studies of nitrogen metabolism. Cul-

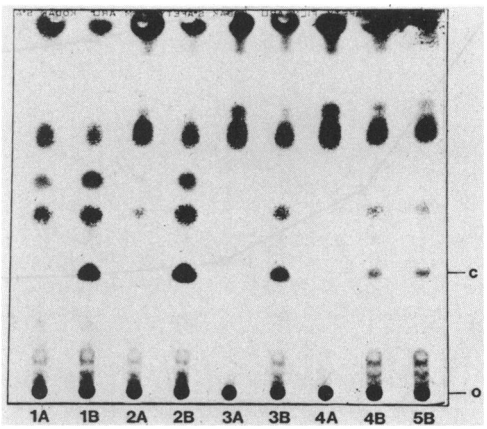

FIG. 3. Thin-layer chromatography of the products of [^{14}C]bicarbonate incorporation into *Anabaena* sp. strain 1F. Nitrate-grown cells were taken from continuous cultures in the absence (lanes A) or presence (lanes (B) of 10 μM AZAT. At 1, 2, 3, 4, and 5 h (lanes 1 through 5, respectively) after the addition of AZAT, 10-ml samples were removed from the culture and 70% ethanol extracts were prepared as described in Materials and Methods. O, Origin; C, [^{14}C]bicarbonate-labeled compound, which had the same R_f as authentic L-[^{14}C]citrulline.

tures of *Anabaena* sp. strain 1F exposed to L-[1-^{14}C]ornithine or [^{14}C]bicarbonate in the presence of AZAT clearly showed that L-[^{14}C]citrulline was formed, which was easily detected by thin-layer chromatography and radioautography. Control experiments showed no significant amount of L-[^{14}C]citrulline formed from [^{14}C]bicarbonate or L-[^{14}C]ornithine in the absence of AZAT. Prior studies demonstrated the enzymatic synthesis of carbamyl phosphate via carbamyl phosphate synthetase and glutaminase in extracts of *A. cylindrica* and *Nostoc muscorum* (9, 14), and the transcarbamylation of ornithine to form citrulline has also been shown (10). During photosynthetic carbon dioxide fixation, L-[^{14}C]citrulline formed from [^{14}C]carbamyl phosphate and ornithine by ornithine transcarbamylase (EC 2.1.3.3) was isolated and identified from cell extracts of *N. muscorum* (11, 15). After 5 min of photosynthesis with radioactive carbon dioxide, the amount of radioactivity in citrulline was as high as 20.9% of the total radioactivity present in 80% ethanol extracts (15). As no free urea or arginine was found in *N. muscorum*, it is unlikely that citrulline is functioning in reactions other than those leading to arginine and urea synthesis (11). The inhibition of photosynthesis in the presence of AZAT, particularly total light-dependent CO_2 fixation, appears to correlate with activation of the incorporation of $H^{14}CO_3^-$ into carbamyl phosphate and citrulline. Although much additional experimentation is required, these results certainly suggest that AZAT profoundly affects carbon metabolism in heterocystous cyanobacteria.

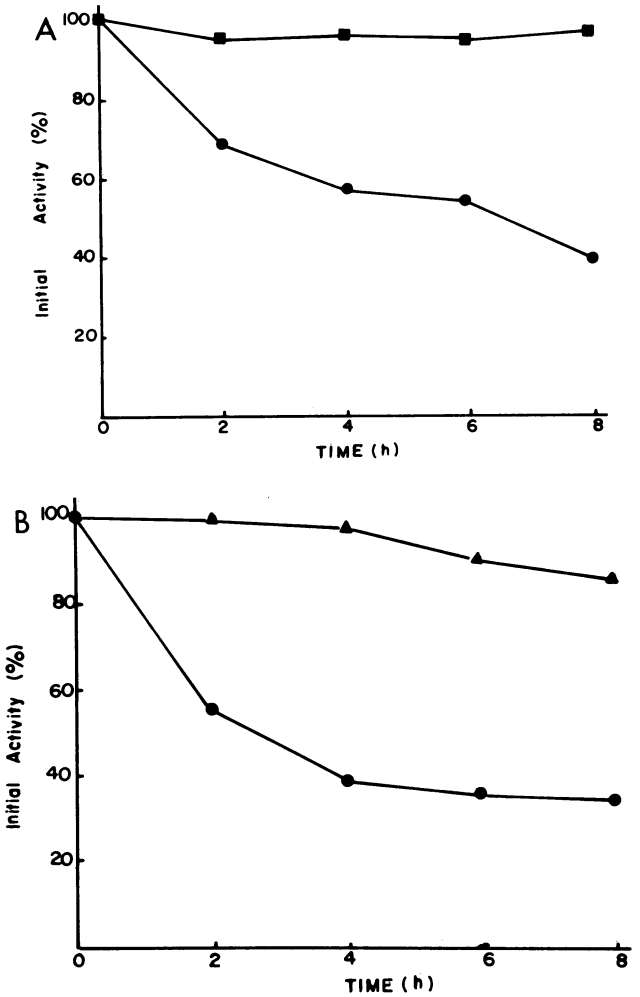

FIG. 4. Effect of AZAT added at time zero on the oxygen evolution activity of nitrate-grown cultures of (A) *Anabaena* sp. strain 1F and (B) *Anabaena* sp. strain CA. Initial oxygen evolution activity was 31.9 nmol of oxygen per mg of chlorophyll per min for *Anabaena* sp. strain 1F and 24.6 nmol of oxygen per mg of chlorophyll per min for *Anabaena* sp. strain CA. Symbols: ■ and ▲, control (no AZAT); ●, 10 μM AZAT.

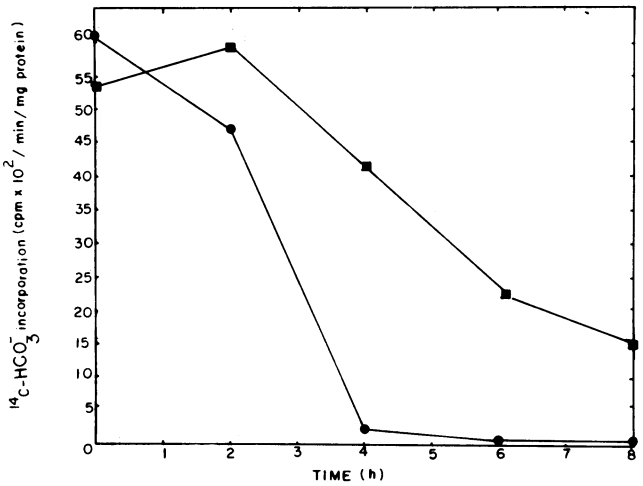

FIG. 5. Effect of AZAT added at time zero on [^{14}C]bicarbonate incorporation of nitrate-grown cultures of *Anabaena* sp. strain 1F (■) and *Anabaena* sp. strain CA (●).

The correlations between primary ammonia assimilation and heterocyst differentiation in *Anabaena* sp. strains 1F and CA indicated that the pool patterns of intracellular amino acids play an important role in the regulation of heterocyst development (5; C. H. Chen, C. Van Baalen, and F. R. Tabita, Abstr. Annu. Meet. Am. Soc. Microbiol. 1984). Our data suggest that amino acids per se are not the initiative factor in triggering heterocyst development. The change in the pool patterns of intracellular amino acids, specifically the ratio of intracellular glutamate to glutamine which can be caused by the presence of amino acid analogs, is an important physiological signal to the cells. In *Anabaena* sp. strain CA, AZAT caused a shortage of glutamate due to the inhibitory effect of this compound on GOGAT (5). This somehow triggers the development of heterocysts, the site of nitrogen fixation (1, 8) under aerobic conditions. However, due to the presence of an alternative (salvage) route of ammonia assimilation, glutamine synthetase-carbamyl phosphate synthetase (and its inherent glutaminase activity [25]), and perhaps other glutaminase activities, vegetative cells of *Anabaena* sp. strain 1F are able to compensate for the loss (or absence) of GOGAT activity caused by the presence of AZAT and regenerate glutamate from glutamine or ammonia without the initiation of heterocyst development.

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