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Novel Mutant of *Anabaena* sp. Strain CA Which Grows on N_2 But Not on Combined Nitrogen

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A mutant has been isolated from *Anabaena* sp. strain CA by treatment with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine, which has the unusual phenotypic characteristic of growth only under N_2-fixing conditions. Growth of the mutant was completely inhibited by NO_3^- or NH_4^+ at concentrations routinely used for growth of the wild type, and sensitivity to NH_4^+ was especially pronounced. The inhibitory effect of NH_4^+ could not be overcome by glutamine, glutamate, or casein hydrolysate. Ammonia had no immediate inhibitory effect on protein synthesis, CO_2 fixation, or O_2 evolution, and the gradual inhibition of C_2H_2 reduction activity by NH_4^+ resembled a repression phenomenon. The glutamine synthetase activity of N_2-fixing cultures appeared normal, yet the mutant was incapable of utilizing exogenous NH_4^+ for growth. Preliminary evidence suggests a possible alteration of glutamine synthetase, which could result in sensitivity to exogenous NH_4^+ by progressive inactivation of the enzyme or repression of its synthesis.

The regulation of nitrogen fixation is a topic of considerable interest, especially in view of the current desire to develop strains of nitrogen-fixing microorganisms which could be used to enhance agricultural productivity. A desired feature of such organisms would be the ability to maintain the synthesis and activity of nitrogenase in the presence of combined nitrogen; thus, a thorough understanding of the factors controlling the expression of nitrogen fixation is required. This has been studied in considerable detail with several types of heterotrophic bacteria (2, 9, 12, 26, 28). The nitrogen-fixing cyanobacteria (blue-green algae) are possibly of even greater significance, since nitrogen fixation in these photoautotrophic organisms is dependent upon light energy and occurs under aerobic conditions. However, the regulation of nitrogen fixation has not been as well studied in cyanobacteria as in heterotrophic bacteria. Ammonia at a concentration of 2 to 3 mM is sufficient to completely repress nitrogenase synthesis in all of the common laboratory strains of heterocystous cyanobacteria, whereas repression by nitrate is often only partial (22). The repression signal for nitrogenase in *Anabaena cylindrica* appears not to be NH_4^+ per se, but some product of NH_4^+ incorporation (24). Recently, Bottomley et al. (1) reported that in *Anabaena* sp. strain CA, considerable nitrogenase activity is expressed indefinitely in medium containing 10 mM NH_4Cl, whereas KNO_3 completely represses nitrogenase synthesis. Thus, this organism appears to show novel characteristics with respect to the regulatory effects of combined nitrogen, with nitrogenase always partially derepressed in the presence of high concentrations of NH_4^+.

The study of regulation of nitrogen fixation in bacteria has been greatly facilitated by the isolation and characterization of numerous mutant strains (8, 15, 25). We have previously reported the isolation of several types of mutants of *Anabaena* sp. strain CA defective in nitrogen fixation (4). We report here the isolation of a mutant with novel phenotypic characteristics, i.e., the inhibition of growth by combined nitrogen, particularly NH_4^+, and the ability to grow only under N_2-fixing conditions. Physiological studies on the inhibitory effect of NH_4^+ suggest an alteration of the regulatory properties of this organism, and preliminary experiments suggest that this might involve glutamine synthetase.

MATERIALS AND METHODS

Organism and culture conditions. The parent strain was the filamentous cyanobacterium *Anabaena* sp. strain CA (ATCC 33047). The medium and growth conditions were as previously described (20). Growth was measured turbidimetrically with a colored glass filter with peak transmission at 660 nm. Dry weights were determined after filtration of cell suspensions through tared Nuclepore filters (0.4 μm) and drying to constant weight in a vacuum oven at 42°C over P_2O_5. Protein was determined by the method of Lowry et al.

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(7) after digestion of samples for 90 min in 0.5 N NaOH at 50°C.

Mutagenesis and selection procedures. Cultures of *Anabaena* sp. strain CA grown in medium minus combined nitrogen were mutagenized by treatment with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine and then broken into single cells by gentle sonication, plated, and incubated as previously described (4). The plates were made with medium containing a low level (34 μM) of L-glutamine and no other combined nitrogen, solidified with 1.0% agar (Difco 0140).

Nitrogenase activity. The acetylene reduction assay (23) was used to measure nitrogenase activity. The assays were performed as previously described (4), except that the tubes were incubated in the same bath as that used for growth of the cultures (39 ± 0.1°C; four fluorescent lamps [F36T12/D/HO], two on either side of the bath at about 15 cm from tubes).

Photosynthesis. Photosynthesis was measured as O_2 evolution using a YSI Clark-type oxygen electrode (no. 5331) mounted in a water-jacketed cell at 39 ± 0.05°C. Samples (1.9 ml) of growing cultures at a density equivalent to about 0.10 to 0.12 mg (dry weight) per ml were placed in the chamber and bubbled for 3 min with 1% CO_2 in air before the chamber was stoppered and measurements were recorded. Changes in electrode current were detected and amplified with a Keithley model 150B microvolt-ammeter. Illumination was provided by a saturating beam from a Standard 500 Junior projector with a 500-W DAY Sylvania bulb operated at 90 V, with two screens inserted between the lamp housing and electrode chamber.

Photosynthesis was also measured as CO_2 fixation by determination of incorporation of $NaH^{14}CO_3$. Radioactive $NaH^{14}CO_3$ (20 μCi; New England Nuclear Corp., Boston, Mass; specific activity, 1 μCi/10 μg) was added to 20 ml of cell suspension in a growth tube which was then returned to growth conditions. Samples (1.0 ml) were removed at intervals and placed in scintillation vials with 0.4 ml of 50% acetic acid. Soluene 350 (1 ml; Packard Instrument Co., Downers Grove, Ill.) was then added to each vial. Radioactivity was counted after addition of 10 ml of Insta-Gel scintillation cocktail (Packard) to each vial.

Assimilation of [*U*-^{14}C]leucine. The assimilation of radioactive leucine was used as an indicator of protein synthesis. To 10 ml of cell suspensions in growth tubes was added 2 μCi of L-[*U*-^{14}C]leucine (Amersham/Searle, Arlington Heights, Ill; specific activity, 2.3 μCi/μg), and the tubes were then returned to growth conditions. At intervals, 0.5-ml samples were removed and filtered through 0.4-μm Nuclepore filters. The filters were washed with growth medium and then placed in 1 ml of Soluene 350 in scintillation vials. The samples were counted after addition of 10 ml of Insta-Fluor scintillation cocktail (Packard).

Glutamine synthetase activity. The transferase activity of glutamine synthetase was measured with the assay of Shapiro and Stadtman (16), based on the formation of γ-glutamylhydroxamate. One unit of activity is defined as the amount of enzyme needed to form one micromole of product per minute. Assays were performed on cell extracts or on whole cells after treatment with toluene (19). For extracts, cells (30 mg [dry weight]) were harvested by centrifugation and washed in buffer containing 10 mM imidazole-hydrochloride and 2 mM $MnCl_2$ (pH 7.0), the same buffer used to suspend cells for toluene treatment for whole-cell assays. The wash was repeated, and the pellet was finally suspended in 5 ml of buffer. This suspension was chilled in ice and sonicated at output setting no. 7 (Branson Sonifier model S125) for three 20-s bursts. The resulting material was centrifuged for 5 min at 15,000 rpm to remove cell debris and then the supernatant was centrifuged at 105,000 × *g* for 20 min in a Beckman Airfuge ultracentrifuge. The high-speed supernatants were pooled and dialyzed for about 18 h against buffer at 5°C.

Extraction and chromatography of amino acid pools. Cells were grown in medium lacking combined nitrogen to approximately 0.6 to 0.7 mg (dry weight) per ml. The suspension was divided into 15-ml portions in separate growth tubes. Filter-sterilized solutions of NH_4Cl or L-methionine-D,L-sulfoximine (MSX) were added at this time. When MSX and NH_4Cl were both added to a tube, the MSX was added first, and the suspension was allowed to stand for 5 min before the addition of NH_4Cl. The tubes were then returned to growth conditions, bubbled with 1.0% CO_2 in air, and incubated for 20 min. The cells were then centrifuged, and the supernatants were discarded. The pellets were heated for 5 min in a boiling water bath. The fluid resulting from this treatment, containing the amino acids released from the cells, was subjected to paper chromatography. Of each sample, 20 μl was applied to Whatman 3 MM paper and developed with *n*-butanol-50% acetic acid (2:1,vol/vol). The solvent front was allowed to move 15 to 18 cm from the origin, and the chromatogram was then dried overnight. Amino acids were stained by briefly dipping the chromatogram in 0.2% ninhydrin in acetone and then heating at 85°C for 5 min.

RESULTS

Selection of the mutant strain. Strain EM18 was selected as a minute colony on a plate containing 34 μM L-glutamine on which wild-type cells had grown to large, well-defined colonies after about 1 week of incubation. It was thought that a colony of this type might represent a glutamine auxotroph whose growth was limited by the small amount of glutamine present. Although it was soon evident that this strain did not require exogenous glutamine for growth and probably could have been selected for in the absence of glutamine, its growth characteristics proved to be extremely unusual.

Growth characteristics. The early growth screening in liquid media revealed that at 39°C, growth was completely inhibited by NO_3^- or NH_4^+ at the concentrations routinely used. Growth occurred only on N_2, at a rate of approximately half that of the wild type (Table 1). The inhibition of growth of EM18 by NO_3^- was found to be a temperature-sensitive phenomenon (Table 1). This table also shows that the organism

TABLE 1. *Growth of mutant strain EM18 and parent strain CA as a function of temperature and nitrogen source*[a]

Strain	Temp (°C)	Growth with N source:		
		N_2	12.6 mM $NaNO_3$	1.0 mM NH_4Cl
EM18	30	12.0	8.9	NG[b]
EM18	35	11.1	22.2	NG[b]
EM18	39	10.4	NG[c]	NG[c]
CA	39	4.7	4.3	4.0

[a] Values given are generation times in hours; each represents the mean of at least three experiments. The inocula were grown in medium minus combined nitrogen.

[b] Indicates slow growth occurring until a culture density indicated by an optical density of 0.20 to 0.25 (about 0.11 to 0.15 mg [dry weight] per ml) was reached, after which growth ceased and cell lysis occurred. Growth did not persist through a second transfer under these conditions.

[c] Indicates no growth.

remained sensitive to NH_4^+ even at lower temperatures, although these cultures did grow from inocula grown on N_2 to a moderate cell density before lysis. Growth at 39°C was inhibited by NH_4Cl at 0.5 mM but not at 0.1 mM (a concentration at which C_2H_2 reduction occurred at a rate comparable to that in an N_2-grown culture). The organism did grow in the presence of up to 5 mM L-glutamine; however, it appeared not to utilize this compound for growth, since heterocysts and nitrogenase activity remained the same as in cultures grown on N_2 (not shown). This phenomenon was also observed with the wild type. The mutant failed to grow in the presence of L-glutamate (1 mM) or casein hydrolysate (100 mg/liter), and no growth occurred when the medium contained either L-glutamine (2 mM) or casein hydrolysate (50 mg/liter) in addition to NH_4Cl (1 mM).

Glutamine synthetase activity. The observation that strain EM18 could not grow by utilizing NH_4^+ or NO_3^- as a nitrogen source (at 39°C) suggested the possibility that the incorporation of NH_4^+ via glutamine synthetase might be impaired in this organism. The activity of this enzyme was accordingly determined in dialyzed extracts of EM18 and compared with that of the wild type (Table 2). There appeared to be virtually no difference in the activity (as measured by the transferase assay) between EM18 and the wild type in N_2-fixing cultures.

Effects of NH_4^+ on protein synthesis, photosynthesis, and nitrogenase activity. The overall inhibitory effect of NH_4^+ on the mutant strain was investigated more closely by attempts to observe the effect of NH_4^+ on major physiological processes. In Fig. 1A it is evident that the addition of NH_4^+ had no immediate effect on protein synthesis (as indicated by [U-^{14}C]leucine incorporation), and Fig. 1B shows that NH_4^+ caused no immediate inhibition of CO_2 fixation.

The absence of an effect of NH_4^+ on photosynthesis is also shown in Fig. 2, for which photosynthesis was measured as O_2 evolution. It should be noted that the control rate of photosynthesis in the mutant grown on N_2 was 126 μl of O_2 per mg (dry weight) per h (average of 4 experiments), whereas that of the wild type was 214 μl of O_2 per mg (dry weight) per h. Also, the control rate of C_2H_2 reduction in the mutant averaged 42.0 nmol of C_2H_4 per mg of protein per min, whereas under the same conditions, that of the wild type averaged 76.9 nmol of C_2H_2 per mg of protein per min. These consistently lower rates of photosynthesis and nitrogenase activity in the mutant correspond to the lower growth rate observed in this strain in comparison with the wild type (Table 1). Although photosynthesis in the mutant was hardly affected by NH_4^+ after 6 h, Fig. 2B clearly shows that NH_4^+ completely inhibited C_2H_2 reduction by this time. This contrasts with the wild type (Fig. 2A), which retained about 25% of its nitrogenase activity in the presence of NH_4^+. Bottomley et al. (1) have shown that strain CA will retain up to 30% of its nitrogenase activity in the presence of 10 mM NH_4^+ even after prolonged subculturing in the NH_4^+-containing medium. The inhibition of C_2H_2 reduction of the mutant by NH_4^+ appears not to be an effect of NH_4^+ per se, since the inhibition was not observed in a culture in which NH_4^+ incorporation via glutamine synthetase was blocked by MSX (Fig. 2B)

Effect of NH_4^+ addition on growth, glutamine synthetase, and free amino acid

TABLE 2. *Glutamine synthetase activity in dialyzed extracts of mutant strain EM18 and parent strain CA*[a]

Growth temp (°C)	Glutamine synthetase activity in:		
	N_2-grown cells		12.6 mM $NaNO_3$-grown cells
	CA	EM18	EM18
39	1.90	2.11	NG[b]
35	1.82	1.86	1.85
30			1.56
30			1.89[c]

[a] Values given are average specific activities (units per milligram of protein) of duplicate determinations. Assays were carried out at the growth temperature, except where otherwise indicated.

[b] NG, No growth under these conditions.

[c] Assayed at 35°C.

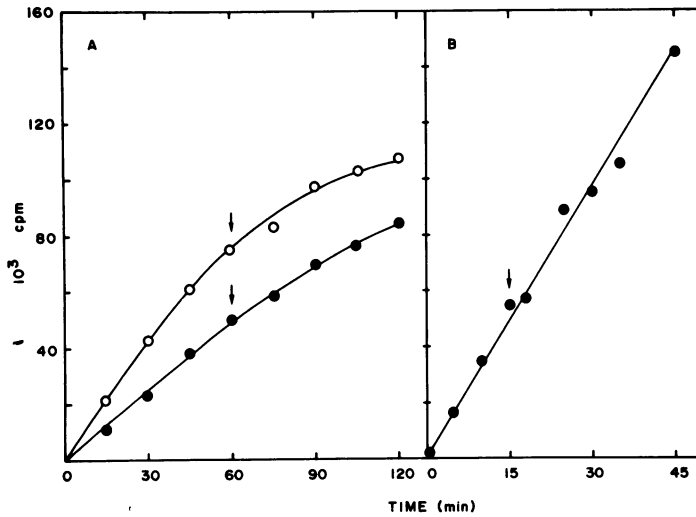


FIG. 1. *(A) Effect of ammonia on incorporation of* L-*[U-*^{14}*C]leucine in the wild-type strain CA* (○) *and the mutant strain EM18* (●). *NH_4Cl (2 mM) was added to culture under growth conditions at the time indicated by the arrow. The culture density in each case was about 0.40 mg (dry weight) per ml; temperature was 39°C. (B) Effect of ammonia on incorporation of $NaH^{14}CO_3$ in mutant strain EM18. NH_4Cl (1 mM) was added at the time indicated by the arrow. The culture density in each case was 0.084 mg (dry weight) per ml; temperature was 39°C.*

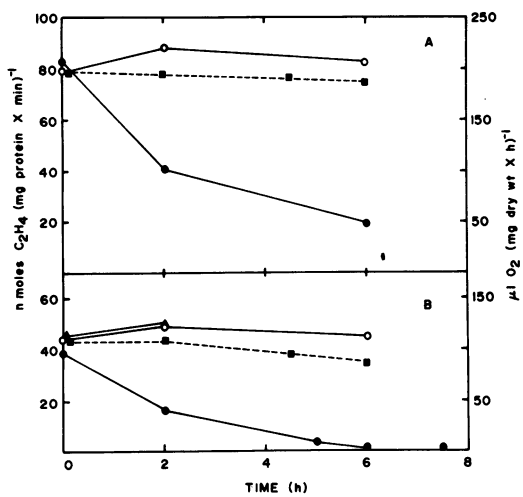


FIG. 2. *Effect of NH_4^+ on C_2H_2 reduction and O_2 evolution in strains CA (A) and EM18 (B). NH_4Cl (2 mM) was added at time zero. The culture density in each experiment was in the range of 0.10 to 0.12 mg (dry weight) per ml. C_2H_2 reduction rate (specific activity) in untreated cells* (○); *cells with NH_4Cl addition* (●); *cells with NH_4Cl plus 10 µM MSX addition* (▲); *photosynthetic O_2 evolution in cells with NH_4Cl addition* (■). *The temperature was 39°C.*

pools of strain EM18. Table 1 indicates that no growth was observed when strain EM18 was inoculated into medium containing NH_4Cl. Figure 3 shows the effect on growth of adding NH_4Cl to a culture of EM18 already growing on N_2. The inhibition of growth was not immediate but occurred over about one generation time. This is consistent with the failure to observe immediate effects of NH_4^+ on major physiological processes. The glutamine synthetase activity was also monitored in whole-cell assays over the time course (Fig. 3).There was an immediate decrease in activity after NH_4^+ addition, and a lowering of the rate of increase in total units of glutamine synthetase until the activity leveled off at about the same time that growth ceased.

For more direct observation of the effects of NH_4^+ on strain EM18 with respect to nitrogen metabolism, the free amino acid pools were extracted and examined by paper chromatography. These experiments were performed at 35 and 39°C to determine possible temperature-sensitive responses. Figure 4, left, shows a comparison of EM18 with the wild type at 35°C. The major spot visible for untreated cells of N_2-grown cultures corresponded to L-glutamate. The predominance of glutamate in the free amino acid pool of cyanobacteria has also been observed by other workers (11, 13). After incubation of N_2-grown cells with NH_4^+, the glutamate spot was reduced, and a new prominent spot corresponding to L-glutamine was observed which probably represents the activity of glutamine synthetase. This interpretation is supported by the finding that MSX inhibited the formation of the glutamine spot. At 35°C, there

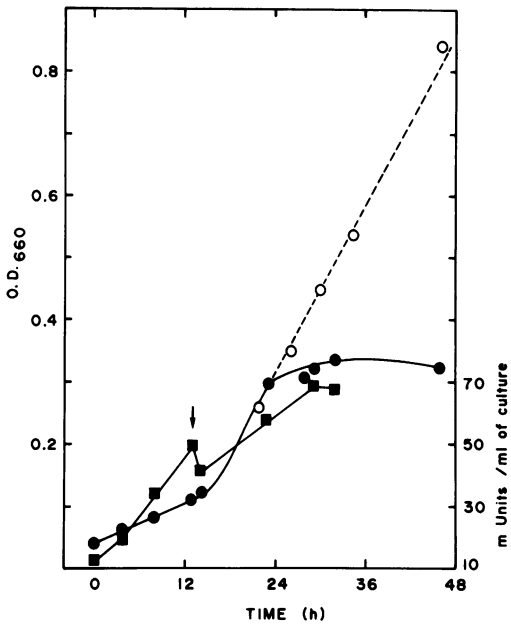
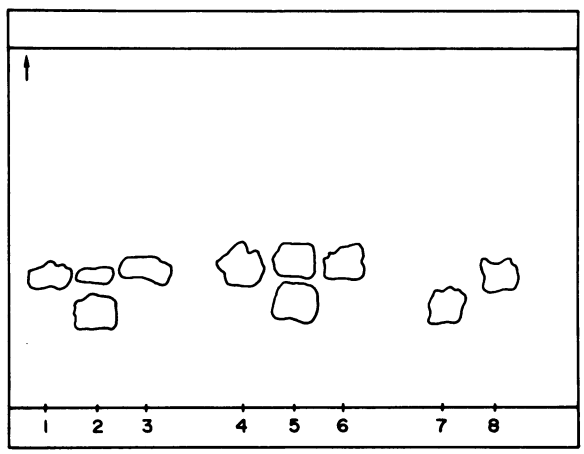


FIG. 3. *Effect of NH_4Cl addition on growth and whole-cell glutamine synthetase activity of strain EM18. NH_4Cl (2 mM) was added at the time indicated by the arrow to a culture of EM18 growing at 39°C in medium minus combined nitrogen. Growth of the culture with NH_4Cl addition (●); growth of the culture with no additon (○); glutamine synthetase activity (whole-cell transferase assay) (■).*

were no indications of any differences between the mutant strain and the wild type; the responses of both strains to NH_4^+ and MSX were identical. The effect of MSX on glutamine synthetase activity in dialyzed extracts was also determined for each strain, and at 35°C the enzyme from both strains showed the same inhibition by MSX (Fig. 4, right). At 39°C, however, the mutant displayed a pronounced difference from the wild type in response to MSX. Figure 5, left, shows that with the mutant, the formation of the glutamine spot upon addition of NH_4^+ was not affected by the same concentration of MSX which completely inhibited this formation in the wild type. This suggests the possibility of an alteration of the glutamine synthetase of EM18 at this temperature. In dialyzed extracts, the enzyme at 39°C from EM18 (as measured by the transferase assay) may be more resistant to MSX than is the enzyme from CA, especially at low MSX concentration (equivalent to the concentration used in the chromatography experiments) (Fig. 5, right). The enzyme from both strains was completely inhibited at higher MSX concentrations.

DISCUSSION

Strain EM18 apparently represents a type of mutant not reported in the literature. The ammonia-sensitive mutant of *Klebsiella aerogenes*



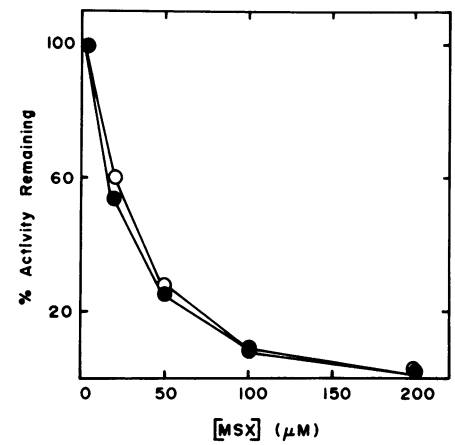


FIG. 4. *(Left) Tracing of original paper chromatogram of amino acids in a water extract of strains CA and EM18 grown at 35°C. Procedures for treatment of cells and for chromatography are described in the text. The line indicated by the arrow represents the solvent front. Cells were grown in medium minus combined nitrogen to a density of 0.6 to 0.7 mg (dry weight) per ml and incubated for 20 min under growth conditions after additions as indicated: 1 and 4, no additions; 2 and 5, 1.0 mM NH_4Cl; 3 and 6, 10 μM MSX plus 1.0 mM NH_4Cl. 1, 2 and 3, Strain CA; 4, 5 and 6, strain EM18; 7, L-glutamine standard, 5 μg; 8, L-glutamate standard, 5 μg. (Right) Inhibition of glutamine synthetase activity by MSX in strains CA (○) and EM18 (●) grown and assayed at 35°C. Assays of transferase activity were performed on dialyzed extracts prepared as described in the text. Assays were initiated by addition of L-glutamine after the extract had incubated in the presence of MSX for 10 min. For CA, 100% activity was 1.82 U/mg of protein; for EM18, it was 1.86 U/mg of protein.*

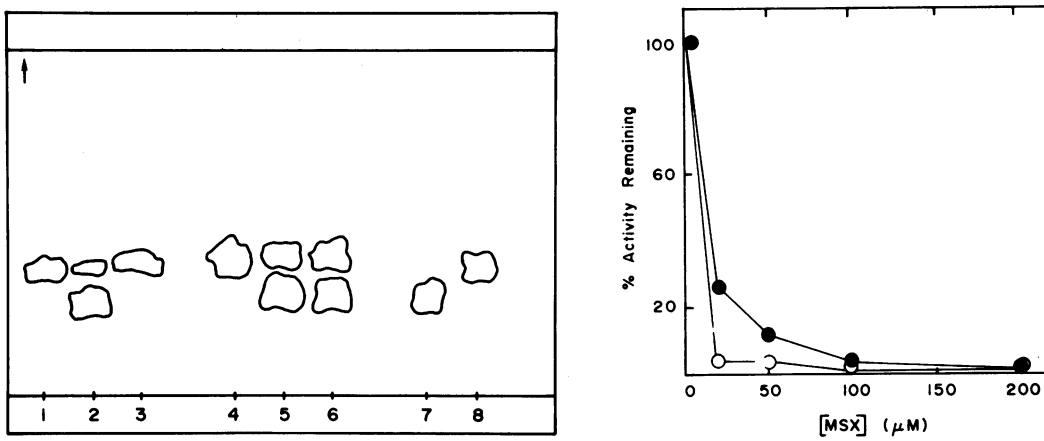


FIG. 5. *(Left) Tracing of original paper chromatogram of amino acids in a water extract of strains CA and EM18 grown at 39°C. Conditions and lanes were as given in the legend to Fig. 4. (Right) Inhibition of glutamine synthetase activity by MSX in strains CA* (○) *and EM18* (●) *grown and assayed at 39°C. Assays of transferase activity were performed on dialyzed extracts prepared as described in the text. Assays were initiated by additon of* L-*glutamine after the extract had incubated in the presence of MSX for 10 min. For CA, 100% activity was 1.90 U/mg of protein; for EM18 it was 2.11 U/mg of protein.*

reported by Struhl and Magasanik (27) was inhibited by 30 mM NH_4^+ but could grow normally on 1 mM NH_4^+, glutamate or histidine. Thus far, the mutant strain EM18 described here has been shown to grow only under N_2-fixing conditions. The overriding phenotypic trait of this strain is its sensitivity to NH_4^+. Presumably, growth occurs on N_2 because the intracellular NH_4^+ concentration under these conditions remains very low. A similar reason for the growth observed on NO_3^- at lower temperatures could be postulated, with the rate-limiting reduction of NO_3^- to NH_4^+ keeping the NH_4^+ concentration low. The inhibiting effect of NH_4^+ does not appear to be a direct effect of NH_4^+ per se on protein synthesis or photosynthesis (Fig. 1 and 2), and thus it is not likely that NH_4^+ is inhibiting growth by acting as an uncoupling agent affecting the supply of ATP for biosynthetic processes (3, 11). There was a complete inhibition of C_2H_2 reduction by NH_4^+ (Fig. 2); however, this inhibition was not a rapid phenomenon like the NH_4^+ inhibition of NO_3^- reduction (6). Therefore, NH_4^+ is probably not acting as an inhibitor of nitrogenase by affecting the redox status of the cell (6) or by causing the formation of a cyanide-enzyme complex (17, 18), as has been postulated for the NH_4^+ effect on NO_3^- reduction. It is also unlikely, according to the kinetics of inhibition, that the inhibition is a feedback response of nitrogenase activity to the presence of combined nitrogen, as seems to be functional in some photosynthetic bacteria (5, 10). Nevertheless, the total inhibition of nitrogenase activity in strain EM18 was the most striking physiological response to NH_4^+ observed. The data suggest that this response is probably a repressive effect of NH_4^+ on nitrogenase synthesis. Nitrogenase synthesis in the wild type is only partially repressed by NH_4^+, with up to 30% of the full activity remaining through many generations of growth in the presence of high concentrations of NH_4^+ (1). Thus, the response of the mutant to NH_4^+ shows a fundamental difference from the wild type in this respect, which could represent a mutation in a regulatory function involving nitrogenase synthesis. This apparently depends upon the incorporation of NH_4^+, since the blocking of glutamine synthetase-mediated NH_4^+ incorporation with MSX prevented the NH_4^+ inhibition of nitrogenase activity (Fig. 2B). The apparent inability of strain EM18 to grow by utilizing exogenous NH_4^+ immediately raised questions concerning the enzymes of nitrogen assimilation in this organism. Certainly, assays of glutamine synthetase indicated no loss in activity of this enzyme as compared with the wild type in N_2-fixing cultures (Table 2). Data on amino acid pools also suggested the presence of an active glutamine synthetase (Fig. 4, left, and 5, left). The question remained as to why the mutant seemed incapable of utilizing exogenous NH_4^+ for growth when N_2 fixation was repressed. We have presented data which give some preliminary indication that the glutamine synthetase of strain EM18 is different from that of the wild type. Although this putative alteration does not seem to result in a loss of glutamine synthetase activity in N_2-fixing cells (as seen in transferase assays), it could reflect a change in regulatory or structural properties ultimately resulting in sensitivity to NH_4^+. We have found

that the response to MSX of cultures in the presence of NH_4^+ can serve as an indicator of possible differences in the glutamine synthetase of the mutant and the wild type. Figure 5, left, strongly suggests that the glutamine synthetase of the mutant is less sensitive to MSX than is that of the wild type, although, as shown in Fig. 5, right, MSX at higher concentrations does still inhibit the enzyme. (The concentration of MSX used in the experiment shown in Fig. 2B was enough to inhibit NH_4^+ incorporation since this amount was much higher in relation to the number of cells present than in the experiments on amino acid pools.) The fact that the data on amino acid pools indicate a temperature-sensitive alteration in the mutant agrees with the observation that the effect of NO_3^- on growth was also temperature sensitive (Table 1). Apparently, the NH_4^+ resulting from NO_3^- reduction was enough to be inhibitory at 39°C but not at 35°C. The difference in effect of MSX on glutamine synthetase in dialyzed extracts of the mutant versus the wild type was less apparent (Fig. 5, right), although some difference was detected. The transferase assay does not measure the physiological reaction of glutamine synthetase (29), and at this time it is not known whether biosynthetic assays would reveal a more pronounced difference. The available data, nevertheless, may be indicative of a subtle alteration in glutamine synthetase of strain EM18 which could result in drastic physiological consequences. The effects on glutamine synthetase activity of adding NH_4^+ to a culture of EM18 growing on N_2 (Fig. 3) appear to be a slowing of the increase in total activity and a leveling off concomitant with the cessation of growth. Rowell et al. (14) observed a partial and slow inactivation of *A. cylindrica* glutamine synthetase by NH_4^+ which could be reversed by addition of a thiol reagent. The glutamine synthetase of strain EM18 might be altered in such a way as to accentuate the sensitivity to NH_4^+, such that inactivation (or repression) by NH_4^+ gradually becomes complete. Although the available data agree with this interpretation, it would be premature at this time to rule out some other possibilities. The glutamine synthetase of the parent strain CA has been purified and studied in some detail (19, 21). We hope to further investigate the properties of glutamine synthetase from strain EM18. This mutant could offer a unique opportunity to study the regulation of nitrogen incorporation in nitrogen-fixing cyanobacteria.

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