The Effects of Structural Analogues of Amino Acids on Ammonium Assimilation and Acetylene Reduction (Nitrogen Fixation) in *Gloeocapsa (Gleothoece) sp. CCAP 1430/3*

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In *Gloeocapsa*, NH_4^+ formed by N_2 fixation was assimilated by the glutamine synthetase–glutamate synthase pathway. The inhibition of acetylene reduction following addition of NH_4^+ to N_2 -fixing cultures was not caused by NH_4^+ itself but was, most probably, related to increased intracellular levels of glutamine.

Analogues of several amino acids inhibited acetylene reduction. Apart from glutamine analogues, which interfered with NH_4^+ assimilation, these analogues probably acted by inhibiting nitrogenase synthesis. Certain analogues of tryptophan and phenylalanine, which have been reported to overcome NH_4^+ inhibition of cell differentiation and, possibly, nitrogenase synthesis in heterocystous cyanobacteria growing on combined nitrogen, did not prevent NH_4^+ from inhibiting acetylene reduction in cultures of *Gloeocapsa*. In contrast, L-methionine-DL-sulphoximine, which similarly counteracts the effect of NH_4^+ on heterocyst differentiation, also prevented NH_4^+ from inhibiting acetylene reduction in *Gloeocapsa*.

INTRODUCTION

In N_2 -fixing cyanobacteria, as in many other organisms, assimilation of NH_4^+ proceeds by the glutamine synthetase (EC 6.3.1.2)–glutamate synthase (EC 1.4.7.1: the cyanobacterial enzyme is ferredoxin-dependent) pathway (see Mifflin & Lea, 1976). Evidence for the operation of this pathway has been provided by enzymological studies (Dharmawardene *et al.*, 1973; Lea & Mifflin, 1975), radioisotopic data (Meeks *et al.*, 1977, 1978) and also by the use of inhibitors of glutamine and glutamate metabolism, such as L-methionine-DL-sulphoximine (Stewart & Rowell, 1975) and azaserine (Meeks *et al.*, 1977). These inhibitors have also proved useful in studying the mechanism whereby exogenously supplied NH_4^+ inhibits nitrogenase (EC 1.18.2.1), the N_2 -fixing enzyme.

Other inhibitors of amino acid metabolism have also been used to investigate N_2 fixation (acetylene reduction) by cyanobacteria. For example, when added to cultures of heterocystous cyanobacteria growing in medium containing combined nitrogen, certain analogues of tryptophan (Bottomley *et al.*, 1980) and phenylalanine (Rogerson, 1979) allowed the differentiation of vegetative cells into heterocysts. Heterocysts are the site of aerobic N_2 fixation in these organisms, but there have been conflicting reports about whether, in the presence of these analogues, nitrogenase activity appears along with cell differentiation (Bothe & Eisbrenner, 1977; Bottomley *et al.*, 1980; Rogerson, 1979). Finally, canavanine, a structural analogue of arginine, has been reported to inhibit nitrogenase synthesis in cultures of *Anabaena flos-aquae* (Bone, 1972).

The unicellular cyanobacterium *Gloeocapsa* (*Gloeotheca*) fixes N_2 aerobically even though it does not possess heterocysts. Consequently, *Gloeocapsa* is a useful organism in which to study the effects of analogues of amino acids on N_2 fixation, because these effects cannot be complicated by cell differentiation. Although in *Gloeocapsa* NH_4^+ is probably assimilated by the glutamine synthetase–glutamate synthase pathway (Gallon *et al.*, 1978; Meeks *et al.*, 1978), there has been no systematic attempt to examine the enzymes of NH_4^+ assimilation in this organism. In this paper, therefore, we report an investigation into the pathways of NH_4^+ assimilation by N_2 -fixing cultures of *Gloeocapsa* and also report the effect on acetylene reduction of inhibitors of NH_4^+ assimilation and structural analogues of various amino acids.

METHODS

Materials. 2-Amino-4-oxo-5-chloropentanoic acid (AOCP) was synthesized from *N*-carboxybenzoxy-L-aspartyl- α -benzyl ester (Cambrian Chemicals, Croydon) as described by Khedouri *et al.* (1966). L-Methionine-DL-sulphoximine (MSX) and azaserine were purchased from Calbiochem, and all other chemicals from Sigma.

Growth of cultures. *Gloeocapsa* sp. CCAP 1430/3 (Culture Centre of Algae and Protozoa, Cambridge) was grown in 151 sterile medium, either free of combined nitrogen (Gallon *et al.*, 1978) or containing 2 mM NH_4Cl (Mullineaux *et al.*, 1980).

Preparation of extracts of *Gloeocapsa*. For the assay of all enzymes except nitrogenase, broken-cell suspensions and cell-free extracts of *Gloeocapsa* were prepared as described by Tözüm & Gallon (1979).

Enzyme assays. N_2 fixation by cultures (10^5 to 10^6 cells ml^{-1}) was measured by the acetylene reduction technique (Tözüm *et al.*, 1977) after 4 h aerobic incubation under illumination at 2500 lx.

Glutamine synthetase (EC 6.3.1.2) was routinely measured in cell-free extracts by the γ -glutamyl transferase assay of Woolfolk *et al.* (1966). The K_m of the enzyme for NH_4^+ was measured using the biosynthetic assay of Shapiro & Stadtman (1970). **Glutamate synthase** (EC 1.4.7.1) was assayed anaerobically as described by Lea & Mifflin (1975) using broken-cell preparations prepared under N_2 . The ferredoxin used in this assay was isolated from *Gloeocapsa* (Gallon *et al.*, 1973). **Glutaminase** (EC 3.5.1.2) activity was measured as described by Bergmeyer (1974) except that the buffer used was 50 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulphonic acid (HEPES)/NaOH, pH 7.5. **Alanine dehydrogenase** (EC 1.4.1.1), **glutamate dehydrogenase** (EC 1.4.1.2) and **glycine dehydrogenase** (EC 1.4.1.10) were assayed by following the NH_4^+ -dependent oxidation of NAD(P)H at 340 nm in the presence of the appropriate 2-oxo acid (Haystead *et al.*, 1973). **Carbamoyl-phosphate synthetase (glutamine-hydrolysing)** (EC 6.3.5.5) and **carbamoyl-phosphate synthetase (NH_4^+)** (EC 6.3.4.16) were assayed by measuring the conversion of $Na_2^{14}CO_3$ [2.5 μCi (92 kBq); 0.33 μmol] to [^{14}C]hydroxyurea following 10 min incubation with a broken-cell suspension (0.5 ml) of *Gloeocapsa* (Levine & Kretschmer, 1971).

Other assays. Extracellular NH_4^+ was measured by the method of Chaney & Marbach (1962) using 1 ml medium removed from 5 ml samples of *Gloeocapsa* (concentrated 10-fold), previously incubated at 25 °C for 24 h under illumination at 2500 lx. Incorporation of radioactivity from $Na_2^{14}CO_3$ into glutamate and glutamine was measured, after 4 h incubation with or without analogues, as described by Gallon *et al.* (1978). Protein was measured as described by Bailey (1962). Cell numbers were measured in a haemocytometer (Tözüm & Gallon, 1979).

RESULTS AND DISCUSSION

The enzymes of NH_4^+ assimilation

Several NH_4^+ -assimilating enzymes were detected in extracts of *Gloeocapsa* sp. 1430/3 (Table 1), glutamine synthetase being the most active under N_2 -fixing conditions. With NH_4^+ as substrate, the K_m of this enzyme was considerably lower than that of either alanine dehydrogenase or glutamate dehydrogenase, the next most active enzymes. In N_2 -fixing cultures, therefore, NH_4^+ is probably assimilated primarily by glutamine synthetase. However, in extracts from cultures growing on NH_4^+ , glutamate dehydrogenase (NADP) was the most active NH_4^+ -assimilating enzyme and may be involved in NH_4^+ assimilation under these conditions.

Under N_2 -fixing conditions, the amino acid dehydrogenases could be involved in NH_4^+ -assimilation, but their low affinity for NH_4^+ suggests that they would be effective only when intracellular NH_4^+ accumulated to a very high level. Both alanine dehydrogenase and

Table 1. Activities of enzymes associated with NH_4^+ assimilation in *Gloeocapsa*

Enzyme	Specific activity [pkat (mg protein) ⁻¹]		K_m for NH_4^+ (M)
	N ₂ -grown cultures	NH_4^+ grown cultures	
Glutamine synthetase	976	341	4.0×10^{-4}
Glutamate synthase	600	ND	ND
Glutamate dehydrogenase (NADP)	167	797	4.2×10^{-2}
Alanine dehydrogenase (NAD)	231	291	6.9×10^{-2}
Glycine dehydrogenase (NAD)	5.67	ND	ND
Carbamoyl-phosphate synthetase (glutamine)	1.35	ND	ND
Carbamoyl-phosphate synthetase (NH_4^+)	0.13	ND	ND

ND, Not determined.

glycine dehydrogenase required NAD as cofactor, and may therefore be a single enzyme. On the other hand, glutamate dehydrogenase required NADP and therefore appears to be distinct from the other dehydrogenases. A similar situation exists in extracts of *Anabaena cylindrica* which were found to contain NAD-dependent alanine and aspartate dehydrogenases, but NADP-dependent glutamate dehydrogenase (Haystead *et al.*, 1973). No aspartate dehydrogenase was found in extracts of *Gloeocapsa*, though high rates of oxidation of NADH occurred in the presence of oxaloacetate, whether or not NH_4^+ was also added; this activity was probably the result of NAD-dependent malate dehydrogenase (EC 1.1.1.37).

Extracts of N₂-fixing cultures of *Gloeocapsa* also contained glutamate synthase which, as in other cyanobacteria (Lea & Mifflin, 1975), required reduced ferredoxin as cofactor. The specific activity of this enzyme was slightly lower than that of glutamine synthetase, but this might be a result of the difficulty of maintaining anaerobic conditions during isolation and assay of glutamate synthase. The observed rates of glutamine synthetase and glutamate synthase activity would be sufficient to assimilate NH_4^+ produced by nitrogenase, which had a maximum specific activity (acetylene reduction) of about 300 pkat (mg protein)⁻¹.

Addition of NH_4^+ to N₂-fixing cyanobacteria leads to a rapid decrease in nitrogenase activity, and it has been suggested that glutamine synthetase or a product of NH_4^+ assimilation is involved in this effect (see Stewart, 1980). One such product is carbamoyl phosphate, which inhibits nitrogenase when added to cultures of cyanobacteria (Lawrie, 1979) including *Gloeocapsa* (Gallon *et al.*, 1978; Gallon, 1980). Carbamoyl-phosphate synthetase activity was found in extracts of *Gloeocapsa* (Table 1). Activity was greater with glutamine as nitrogen donor than with NH_4^+ , in contrast to the findings of Lawrie (1979) with *A. cylindrica*. Since the glutamine-dependent carbamoyl phosphate synthetase of *Escherichia coli* was active with both NH_4^+ and glutamine (Trotta *et al.*, 1973), both carbamoyl-phosphate synthetase activities in *Gloeocapsa* may be due to a single, glutamine-dependent, enzyme. No glutaminase was detected in extracts of *Gloeocapsa*, so the activity of glutamine-dependent carbamoyl phosphate synthetase was not the result of the combined activities of glutaminase and carbamoyl synthetase (NH_4^+).

Inhibitors of glutamine synthetase

L-Methionine-DL-sulphoximine (MSX) is reported to inhibit glutamine synthetase irreversibly, probably by attachment to both the glutamate and the NH_4^+ binding sites (Meister, 1968). 5-Hydroxylysine is a reversible inhibitor of glutamine synthetase and

Table 2. *Effect of inhibitors of NH_4^+ assimilation on acetylene reduction by *Gloeocapsa**

Cells were collected from batch cultures during the late exponential phase of growth (6 to 10 d after inoculation). They were then incubated aerobically in the light for 4 h with 5 mM-HEPES/NaOH buffer, pH 7.5, and the compounds indicated. Acetylene was added to 2% (v/v) and the rate of acetylene reduction was measured over the following 60 min. The rates of acetylene reduction are typical; the percentages are the means of at least 12 observations.

Addition	Acetylene reduction [pmol min^{-1} (10^6 cells) $^{-1}$]	Percentage of uninhibited activity
None	24.3	100
2 mM-MSX	16.5	68
2 mM-5-Hydroxylysine	14.9	61
2 mM-AOCP	7.3	30
0.2 mM-AOCP	11.4	47
20 μM -AOCP	16.5	68
2 mM-Azaserine	2.8	12
0.2 mM-Azaserine	17.0	70
2 mM-Albizziine	2.2	9
2 mM-S-Carbamoyl cysteine	2.2	9
2 mM-Glutamic acid- γ -hydrazide	73.3	301
2 mM-Glutamine	14.9	61
2 mM-Glutamate	20.2	83
2 mM-Methionine	19.7	81

shows kinetics of competitive inhibition (Rabinowitz *et al.*, 1957; Stewart, 1980). Both 2 mM-MSX and 2 mM-5-hydroxylysine inhibited glutamine synthetase by 95% in extracts of *Gloeocapsa*. N_2 -fixing cultures excreted NH_4^+ when incubated with 2 mM-MSX [$12.5 \text{ pmol min}^{-1}$ (10^6 cells) $^{-1}$] or 2 mM-5-hydroxylysine [$4.16 \text{ pmol min}^{-1}$ (10^6 cells) $^{-1}$]. This suggests that glutamine synthetase catalyses the initial step of NH_4^+ assimilation in *Gloeocapsa* and is further evidence for the operation of the glutamine synthetase–glutamate synthase pathway under N_2 -fixing conditions.

Acetylene reduction was slightly inhibited by 2 mM-MSX or 2 mM-5-hydroxylysine (Table 2). It is not clear whether simultaneous addition of 2 mM-glutamine prevented these inhibitions, because glutamine itself inhibited acetylene reduction (Table 2). Inhibition of acetylene reduction by 2 mM-MSX was prevented by addition of 2 mM-methionine. Incubation for 4 h with 2 mM- NH_4Cl markedly inhibited acetylene reduction, but addition of 2 mM-MSX or 2 mM-5-hydroxylysine greatly decreased the effect of NH_4^+ (Table 3). Indeed, if allowance is made for the inhibitory effects of MSX and 5-hydroxylysine themselves (Table 2), these compounds almost completely prevented NH_4^+ from inhibiting acetylene reduction.

Glutamine synthetase in cultures of other cyanobacteria was inhibited by MSX at concentrations much lower than 2 mM (Stewart & Rowell, 1975; Meeks *et al.*, 1978). However, in *Gloeocapsa*, 20 μM - or 0.2 mM-MSX failed to prevent NH_4^+ from inhibiting acetylene reduction, probably because MSX may be poorly assimilated by *Gloeocapsa*. This view is supported by the observation that simultaneous addition of either 2 mM-glutamine or 2 mM-methionine markedly lowered the effect of MSX in preventing inhibition by NH_4^+ (Table 3), probably by interfering with the uptake of MSX (Meins & Abrams, 1972).

That inhibitors of glutamine synthetase prevented NH_4^+ from inhibiting acetylene reduction in *Gloeocapsa* suggests that NH_4^+ itself was not the inhibitor and that a product of NH_4^+ assimilation may have been involved. Addition of glutamine, itself an early product of NH_4^+ assimilation, only slightly inhibited acetylene reduction (Table 2) but glutamine may not be readily assimilated by *Gloeocapsa*. Alternatively, carbamoyl phosphate, formed from glutamine, could be the inhibitor (Gallon, 1980).

Table 3. *Amino acid analogues and the effect of NH_4^+ on acetylene reduction by *Gloeocapsa**

The assays were done as described for Table 2. The rates of acetylene reduction are typical; the percentages are the means of at least 12 observations. The figures in parentheses show these percentages after correction for the effect of the analogue itself (see Table 2).

Addition*	Acetylene reduction [pmol min^{-1} (10^6 cells) $^{-1}$]	Percentage of uninhibited activity
None	17.6	100
2 mM- NH_4Cl	3.9	22
2 mM- NH_4Cl + 2 mM-MSX	9.7	55 (81)
2 mM- NH_4Cl + 2 mM-MSX + 2 mM-glutamine	5.1	29
2 mM- NH_4Cl + 2 mM-MSX + 2 mM-methionine	5.8	33
2 mM- NH_4Cl + 2 mM 5-hydroxylysine	9.7	55 (90)
2 mM- NH_4Cl + 2 mM-AOCP	2.6	15 (50)
2 mM- NH_4Cl + 0.2 mM-AOCP	4.4	25 (53)
2 mM- NH_4Cl + 20 μM -AOCP	5.3	30 (44)
2 mM- NH_4Cl + 0.2 mM-azaserine	0.9	5 (7)

* The following additions were without effect, at 2 mM, on the inhibition of acetylene reduction by NH_4^+ : 7-azatryptophan, 4-fluorotryptophan, 5-methyltryptophan, canavanine, azetidine-2-carboxylate, 4-fluorophenylalanine and β -2-thienylalanine.

Analogues of glutamine

Azaserine and albizziine have both been reported to inhibit glutamate synthase, the second enzyme in the pathway of NH_4^+ assimilation (Lea & Norris, 1976; Mifflin & Lea, 1976). Azaserine (2 mM) completely inhibited glutamate synthase in extracts and, when added to cultures incubated with $\text{Na}_2^{14}\text{CO}_3$, caused an accumulation of [^{14}C]glutamine [from 16 to 207 Bq (10^6 cells) $^{-1}$] at the expense of [^{14}C]glutamate [which decreased from 176 to 7 Bq (10^6 cells) $^{-1}$]. This is consistent with the operation of the glutamine synthetase-glutamate synthase pathway in *Gloeocapsa*.

Although azaserine, albizziine and *S*-carbamoyl cysteine are analogues of glutamine, they neither significantly inhibited glutamine synthetase *in vitro*, nor caused excretion of NH_4^+ when added to a N_2 -fixing culture. At 2 mM, azaserine, albizziine or *S*-carbamoyl cysteine severely inhibited acetylene reduction by cultures of *Gloeocapsa* (Table 2). Although 0.2 mM-azaserine was less inhibitory (Table 2), it exacerbated the effect of added NH_4^+ (Table 3). Addition of 2 mM-glutamine, simultaneously with analogue (2 mM), had little effect on the inhibition of acetylene reduction, nor was the effect of azaserine prevented by addition of 2 mM-glutamate (Table 4). This suggests that inhibition of glutamate synthase probably affects acetylene reduction by causing an increased intracellular concentration of glutamine (or related product), rather than by depleting intracellular glutamate. If this is so, then it can be predicted that addition of MSX, which would abolish accumulation of glutamine, should prevent azaserine from inhibiting acetylene reduction. Simultaneous addition of 2 mM-MSX with 2 mM-azaserine caused less inhibition of acetylene reduction than that caused by azaserine alone (Table 4), so the effect of azaserine, and, probably, NH_4^+ , on acetylene reduction by *Gloeocapsa* is related to an increase in the intracellular concentration of glutamine or of a related metabolite such as carbamoyl phosphate.

Unlike azaserine, 2-amino-4-oxo-5-chloropentanoic acid (AOCP) is an inhibitor of glutamine-dependent carbamoyl phosphate synthetase (Khedouri *et al.*, 1966). Because it is an analogue of glutamine, AOCP probably inhibits glutamate synthase, but whilst addition of azaserine might result in an increased intracellular concentration of both glutamine and carbamoyl phosphate, AOCP would not allow carbamoyl phosphate to accumulate. Like

Table 4. *Effects of glutamine and glutamate on the inhibition of acetylene reduction by analogues of glutamine*

The assays were done as described for Table 2. The rates of acetylene reduction are typical; the percentages are the means of at least 12 observations. The figures in parentheses show these percentages after correction for the effect of the second addition (see Table 2).

Addition	Acetylene reduction [$\mu\text{mol min}^{-1}$ (10^6 cells) $^{-1}$]	Percentage of uninhibited activity
None	22.2	100
2 mM-Azaserine	2.4	11
2 mM-Azaserine + 2 mM-glutamine	3.9	18 (29)
2 mM-Azaserine + 2 mM-glutamate	3.1	14 (17)
2 mM-Azaserine + 2 mM-MSX	10.9	49 (72)
2 mM-Albizziine	2.0	9
2 mM-Albizziine + 2 mM-glutamine	2.2	10 (16)
2 mM-S-Carbamoyl cysteine	2.0	9
2 mM-S-Carbamoyl cysteine + 2 mM glutamine	2.1	9 (16)

Table 5. *Effect of glutamic acid- γ -hydrazide on acetylene reduction*

Where cells were used, they were collected from batch cultures of *Gloeocapsa* after 6 to 10 d growth. After incubation for 4 h in 150 ml Erlenmeyer flasks, the flasks were sealed, acetylene was added, where indicated, to 2% (v/v) and, following a further 60 min incubation, 1 ml of the gas phase was subjected to gas chromatography. The results are the means of five separate observations.

Incubation*	Ethylene produced ($\mu\text{mol min}^{-1}$ ml $^{-1}$)
<i>Gloeocapsa</i> culture + C_2H_2	8.3
<i>Gloeocapsa</i> culture, no C_2H_2	0
Distilled water + C_2H_2	0
Distilled water, no C_2H_2	0
<i>Gloeocapsa</i> culture + 2 mM-glutamic acid- γ -hydrazide + C_2H_2	25.0
<i>Gloeocapsa</i> culture + 2 mM-glutamic acid- γ -hydrazide, no C_2H_2	3.7
Distilled water + 2 mM-glutamic acid- γ -hydrazide + C_2H_2	18.5
Distilled water + 2 mM-glutamic acid- γ -hydrazide, no C_2H_2	1.0

* The volume of culture or distilled water was 50 ml in each case.

azaserine, 2 mM-AOCP did not inhibit glutamine synthetase but markedly inhibited acetylene reduction by cultures of *Gloeocapsa* (Table 2). This suggests that carbamoyl phosphate is probably not the inhibitor. Furthermore, because azaserine inhibits many metabolic reactions involving glutamine (Buchanan, 1973), the inhibitor may well be glutamine itself. However, this has not been demonstrated. Unlike the other glutamine analogues, AOCP may have reversed, slightly, the effect of NH_4^+ on acetylene reduction by *Gloeocapsa* (Table 3). However, this effect of AOCP, though consistent, was never great and was only really apparent after correction for the inhibition caused by AOCP itself. Such correction may not be valid, so it would be unwise to attach much significance to these observations.

At 2 mM, glutamic acid- γ -hydrazide, another glutamine analogue, apparently markedly stimulated acetylene reduction by *Gloeocapsa* (Table 2). However, detailed examination of the effect of this analogue (Table 5) showed that a compound chromatographically indis-

Table 6. *Effect of several amino acids and their analogues on acetylene reduction by Gloeocapsa*

The assays were done as described for Table 2. The rates of acetylene reduction are typical; the percentages are the means of at least 12 observations.

Addition	Acetylene reduction [$\mu\text{mol min}^{-1}$ (10^6 cells) $^{-1}$]	Percentage of uninhibited activity
None	21.5	100
2 mM-Tryptophan	19.1	89
2 mM-7-Azatryptophan	10.3	48
2 mM-4-Fluorotryptophan	2.4	11
2 mM-5-Methyltryptophan	7.5	35
2 mM-Phenylalanine	18.7	87
2 mM-4-Fluorophenylalanine	7.6	35
2 mM- β -2-Thienylalanine	1.9	9
2 mM-Proline	21.3	99
2 mM-Azetidine-2-carboxylate	9.5	44
2 mM-Arginine	18.1	84
2 mM-Canavanine	15.1	70

tinguishable from ethylene was produced when 2 mM-glutamic acid- γ -hydrazide was incubated, with or without acetylene, in the presence or absence of a culture of *Gloeocapsa*. Glutamic acid- γ -hydrazide may catalyse reduction of acetylene to ethylene but may also itself react in aqueous solution to give a compound that behaves like ethylene during gas chromatography. Although the nature of this compound has not been investigated, it may be hydrazine; a similar apparent production of ethylene has been observed with solutions of isonicotinyl hydrazide (J. R. Gallon, unpublished observations). The stimulation of acetylene reduction by glutamic acid- γ -hydrazide (Table 2) is, therefore, an artefact, and the effect, if any, of this analogue on N_2 -fixation is not known.

The effects of inhibitors of NH_4^+ assimilation on acetylene reduction support the enzymic data that suggest that fixed N_2 is assimilated in *Gloeocapsa* by the glutamine synthetase-glutamate synthase pathway. Furthermore, they confirm that the inhibitory effect of NH_4^+ on acetylene reduction, which is exerted through an inhibition of nitrogenase synthesis (Gallon, 1980) is caused not by NH_4^+ but by a product of its assimilation. The nature and mode of action of the inhibitor are not clear, but it may be glutamine rather than carbamoyl phosphate, as previously suggested (Gallon, 1980). In some N_2 -fixing organisms (see, for example, Stewart, 1980) glutamine synthetase itself may be involved in the inhibition of nitrogenase synthesis, but there is, so far, no evidence for this in *Gloeocapsa*.

Other analogues

At 2 mM, analogues of tryptophan, phenylalanine and proline all markedly inhibited acetylene reduction by *Gloeocapsa* (Table 6). In contrast to the findings of Bone (1972) with *A. flos-aquae*, 2 mM-canavanine had only a slight effect on acetylene reduction. It is possible that canavanine is not easily assimilated by *Gloeocapsa*.

None of these analogues prevented 2 mM- NH_4^+ from inhibiting acetylene reduction by *Gloeocapsa* (Table 3, footnote). 7-Azatryptophan, 4-fluorotryptophan (Bottomley *et al.*, 1980) and β -2-thienylalanine (Rogerson, 1979) have been reported to overcome the inhibition of heterocyst differentiation in filaments of heterocystous cyanobacteria growing on a source of fixed nitrogen. That none of these analogues prevented NH_4^+ from inhibiting acetylene reduction in *Gloeocapsa* suggests that their effect on heterocyst differentiation is probably direct and not related to any effect on nitrogenase. In contrast, the effect of MSX, which also prevents combined nitrogen from inhibiting heterocyst differentiation (Stewart &

Rowell, 1975), may be related to an effect on nitrogenase, because MSX overcame the NH_4^+ -mediated inhibition of acetylene reduction in *Gloeocapsa*.

In contrast to the effect of glutamine on its analogues, the inhibition of acetylene reduction by the analogues listed in Table 6 was prevented, either in part (tryptophan, proline) or completely (phenylalanine, arginine), by addition (2 mM) of the appropriate amino acid. These analogues probably exert their effect on acetylene reduction by interfering with the synthesis of proteins, including nitrogenase and, indeed, 7-azatryptophan, 4-fluorophenylalanine or azetidine-2-carboxylate, each at 2 mM, appeared to inhibit nitrogenase synthesis almost completely, as measured in the system described by Gallon (1980). Because synthesis of nitrogenase is essential in *Gloeocapsa*, in order to balance inactivation of the enzyme by oxygen (Gallon, 1980), interruption of nitrogenase synthesis would, during 4 h aerobic incubation, result in the marked decrease in nitrogenase activity observed in the presence of these analogues (Table 6).

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