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Effects of some amino acid analogues on growth and heterocyst formation in the blue-green alga *Nostoc linckia*

J K LADHA* and H D KUMAR
Department of Botany, Banaras Hindu University, Varanasi 221 005
*Present address: Department of Biological Sciences, University of Dundee, Dundee DDI HN (U K)

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Abstract. The effects of two amino acid analogues, viz., L-methionine-DL-sulphoximine and L-methyl-DL-methionine on growth, heterocyst differentiation and nitrogen fixation in the blue-green alga *Nostoc linckia* have been studied with special reference to heterocyst spacing pattern. L-methionine-DL-sulphoximine strongly inhibited growth but produced an unusual number of heterocysts with changed heterocyst spacing pattern in both nitrogen-free and ammonium-containing media. L-methyl-DL-methionine was less effective than L-methionine-DL-sulphoximine. An attempt was also made to counteract the toxic effects of these analogues by supplying amino acids. Glutamine and methionine reversed the inhibitory effect of L-methionine-DL-sulphoximine while only methionine reversed the inhibitory effect of L-methyl-DL-methionine. Production of changed heterocyst spacing pattern in nitrogen-free and ammonium-containing media when supplemented with L-methionine-DL-sulphioximine suggests that ammonia may not be the inhibitor of heterocyst spacing pattern.

Keywords. L-methionine-DL-sulphoximine; L-methyl-DL-methionine; heterocyst; *Nostoc linckia*.

1. Introduction

Amino acid analogues produce inhibitory effects in a number of ways on growth and metabolism of microorganisms. They can substitute for a normal amino acid in proteins which in turn may lead to formation of partially active or inactive enzymes (Richmond 1962). In bacteria, amino acid analogues have been extensively employed for isolation of mutants to trace amino acid biosynthetic pathways and in investigations on the regulation of ammonia-assimilation. L-methionine-DL-sulphoximine (MetSo), an analogue of Met and Glu, has been widely used to study the regulation of nitrogenase and the primary amination in some bacteria (Brenchley 1973; Gordon and Brill 1974; Veale and Brenchley 1974) and in the blue-green alga *Anabaena cylindrica* (Stewart and Rowell 1975). Only a few reports are available on the effects of amino acids and their analogues in blue-green algae (Fogg *et al* 1973).

We have studied the effect of some amino acids, viz., Gln, Glu, Met, His and two analogues of amino acids, viz., (MetSo) and l-methyl-DL-methionine (MeMet), on growth, heterocyst differentiation and nitrogen fixation with special emphasis on heterocyst spacing pattern in the blue-green alga *Nostoc linckia*, and attempted to counteract the inhibitory effects by natural amino acids.

2. Materials and methods

The organism used in this study was the filamentous, heterocystous and spore-forming blue-green alga *Nostoc linckia*. Its axenic and clonal culture was maintained in Allen and Arnon's medium with or without combined nitrogen (1 mM NH_4Cl) at 28±2°C and a light intensity of 1300-1400 lx for 12 h daily as described previously (Ladha and Kumar 1977a).

Growth was measured in a Bausch and Lomb Spectronic 20 colorimeter at 660 nm. Ammonia estimation was carried out by Nessler's reaction. Heterocyst-free culture of *N. linckia* was obtained by repeated subculturing of the alga in medium containing ammonium chloride (1 mM).

Amino acids Gln, Met, His, and Glu and their analogues (MetSo and MeMet) supplied by Sigma Ltd., St. Louis, MO, USA were freshly prepared in Allen and Arnon's nitrogen-free medium and sterilized by membrane filtration.

3. Results

Figures 1, 2 and 3 show the growth characteristics and heterocyst production in nitrogen-free and ammonium-medium with different concentrations of MetSo and MeMet. Different amounts of analogues were added at the time of inoculation. At 1 μM MetSo was inhibitory to growth, but permitted maximal production of heterocysts. The increase in heterocyst frequency was accompanied by alterations in the heterocyst spacing pattern and heterocysts were formed in chains in both nitrogen-free as well as in ammonium-containing media. Unlike MetSO, complete inhibition of growth occurred at a concentration of MeMet higher than 1 mM and its effect on heterocyst production was slight if at all. In presence of MeMet (0·1-1·0 mM) the vegetative cells became abnormal with tumour-like bulgings. The frequency of these abnormal cells increased when NH_4Cl was added to the medium. The effects of these two analogues on the uptake of ammoniacal nitrogen

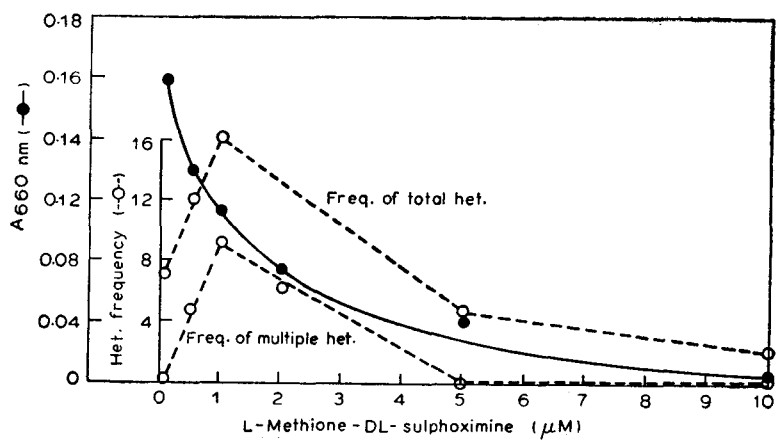

Figure 1. Effects of different concentrations of MetSo on growth and heterocyst frequency of *Nostoc linckia* in nitrogen free medium.

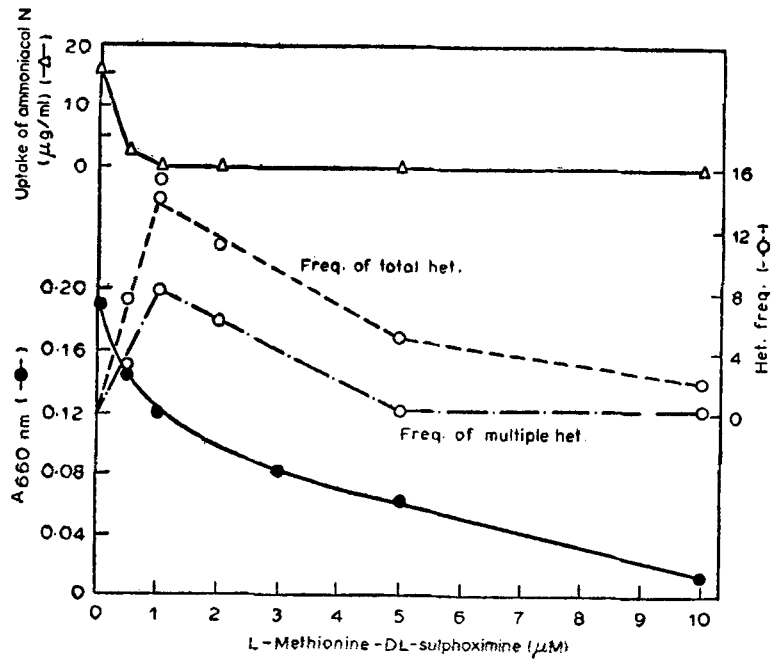

Figure 2. Effects of different concentrations of MetSo on growth, heterocyst frequency and uptake of ammoniacal-nitrogen in ammonium-containing medium.

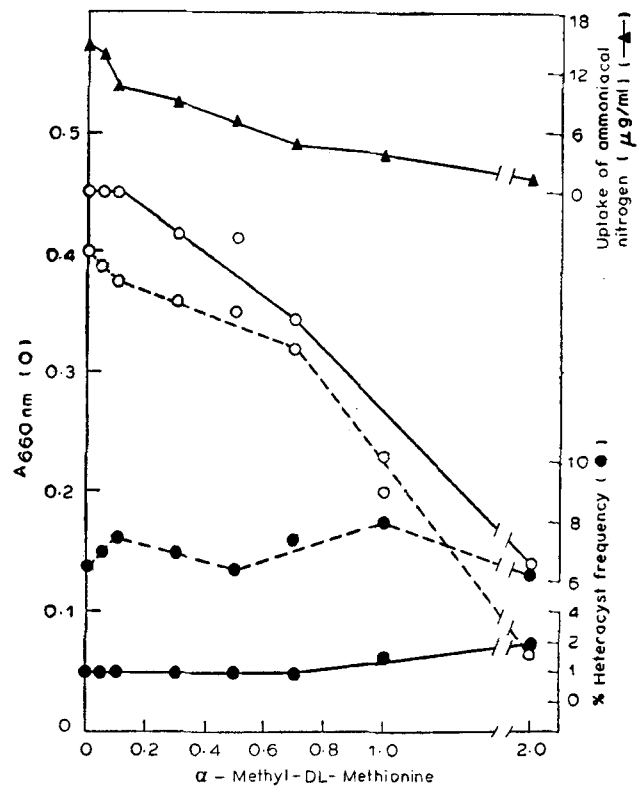

Figure 3. Effects of different concentrations of MeMet on growth and heterocyst frequency in nitrogen-free and ammonium containing media.

by the alga are shown in figures 2 and 3. MetSO and MeMet (at 1 μM and 1 mM respectively) almost completely inhibited the uptake of ammoniacal nitrogen.

In order to study the reversibility of the inhibitory effects of MetSo and MeMet amino acids, viz., Gln, Met, Glu and His were employed. Figure 4 shows the effect of amino acids on growth and heterocyst frequency. Figure 5 represents the time course of growth response at a fixed concentration (500 μM) of each amino acid in the absence of analogues. Gln and Met stimulated while His slightly inhibited growth; Glu was toxic and caused rapid lysis of cells at higher concentrations (>300 μM). Glu and Gln markedly inhibited heterocyst production. At 300-400 μM practically no heterocysts were produced. Met stimulated heterocyst production up to 400 μM concentration.

The ability of different amino acids to overcome the inhibition caused by the analogues is shown in figures 6 and 7. The amino acids (500 μM) were added, 4 days after inoculation, into the culture incubated with MetSo (5 μM) or MeMet (2 mM). Gln and Met counteracted MetSo-inhibition although Met was less effective. Glu acid and His were slightly effective. The inhibitory effect of MeMet was reversed only by Met.

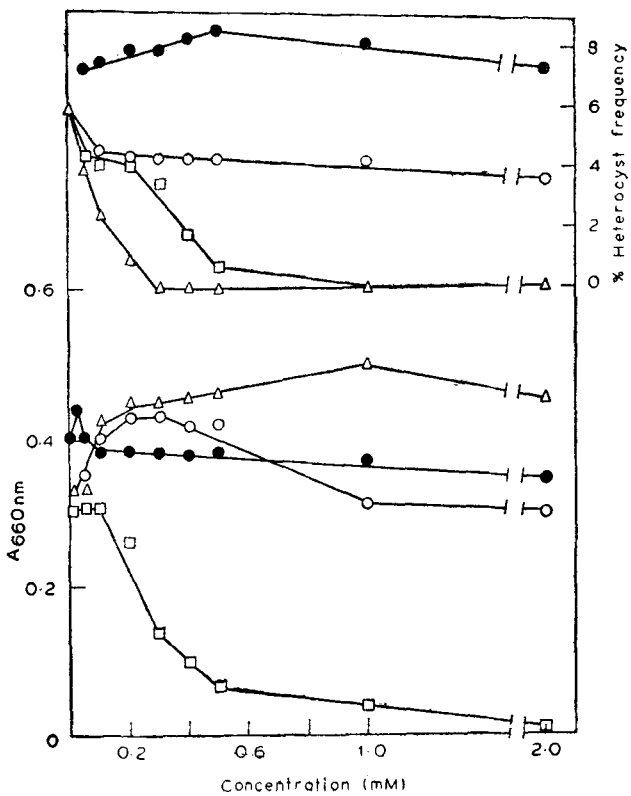

Figure 4. Effects of different concentrations of glutamine, glutamate, methionine and histidine on growth and heterocyst frequency (growth was recorded after 7 days).

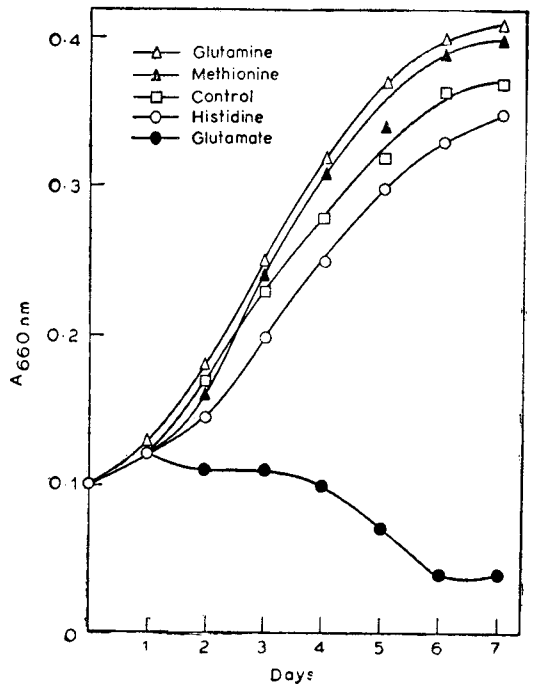

Figure 5. Growth response in nitrogen-free medium to glutamine, glutamate, methionine and histidine (500 μM).

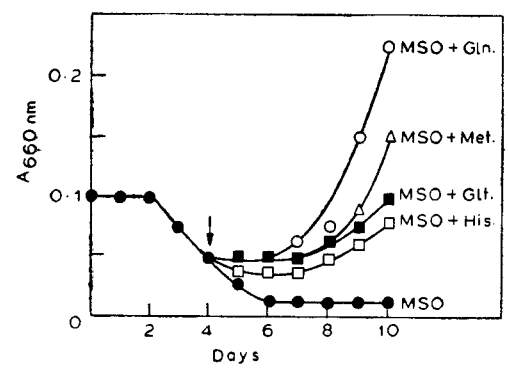

Figure 6. The effect of amino acids (500 μM) in reversing the action of MetSo (5μM); (MSO was added on the 4th day of inoculation).

4. Discussion

The heterocyst spacing pattern seems to be governed by an inhibitory ' diffusible ' substance, produced in the developing heterocysts and transported along the sides (Wilcox *et al* 1973). The nature of this diffusible substance is not known. The earlier view of Fogg (1949) that ammonia (but not its derivative) may be a regulator of heterocyst spacing is not correct because the pattern of heterocyst spacing in *Nostoc* and *Anabaena* emerges even before the appearance of nitrogenase activity

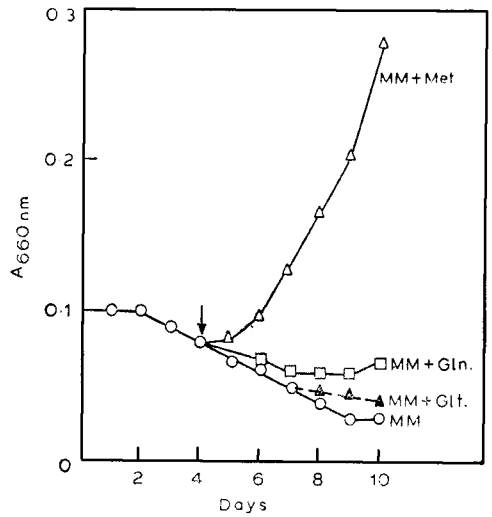

Figure 7. The effect of amino acids (500 μM) in reversing the action of MeMet (5 mM); (MeMet added on the 4th day of the inoculation).

(Kulasooriya *et al* 1972). This is further supported by observation on *het*$^+$ *nif*$^-$ mutants of *Anabaena cylindrica* and *Nostoc linckia* (Wilcox *et al* 1975; Ladha and Kumar 1977b), which did not fix nitrogen and yet produced heterocysts in the usual spacing pattern as in wild-types. Stewart and Rowell (1975) studied the effect of MetSo on *Anabaena cylindrica* and showed that on the one hand it caused starvation of Gln and its associated enzymes (glutamine synthetase, and glutamate synthase) while MetSo treatment led to an accumulation of ammonia into the medium. MetSo also relieved the inhibitory effect of exogenous ammonia on nitrogenase activity and heterocyst production. We have also observed the formation of heterocyst with altered spacing pattern both in nitrogen-free and ammonium-containing media. This strongly supports the view that ammonia *per se* is not regulating heterocyst spacing, but instead it could be Gln or Glu. Recently Wolk and his group (Thomas *et al* 1975 and Wolk *et al* 1976) have identified Glu and Gln as the first and second organic products of nitrogen fixation. Dharmarwardene *et al* (1973) showed that the specific activity of glutamine synthetase in isolated heterocysts is 1·7 times greater than in vegetative cells. Thomas *et al* (1975) have concluded that there is no significant difference in the specific activity of glutamine synthetase in heterocysts compared to vegetative cells, while the specific activity of glutamate synthetase is more in vegetative cells than in the heterocysts. Therefore, it appears that glutamine synthesized in heterocysts is transported to vegetative cells, where Glu is synthesized. This also does not indicate a mobile nature of Glu. In present study we have found that glutamate is toxic to growth and hence the likelihood of glutamate synthetase or Glu acting as a regulator seems unlikely.

All available indications therefore suggest that Glu may be the inhibitor that moves from cell to cell. To further check this possibility it requires to isolate a Gln-requiring mutant.

Reversibility of MetSo inhibition by Glu suggests that MetSo treatment leads to Glu deficiency, but the resumption of growth in the presence of MetSo and methionine indicates that glutamine synthetase, glutamate synthetase or a product of their re-

actions may not necessarily be the repressor of heterocyst differentiation. Therefore, we cannot rule out the possibility of pleiotropic effects of MetSo. To check whether or not Met or enzymes of its metabolism might be involved in heterocyst regulation, we have also studied the effect of another analogue of Met, viz. MeMet. It was found that the inhibitory effect of this analogue was reversed by Met but not by Gln or Glu. Moreover, unlike MetSo, treatment with MeMet did not result in any drastic change in heterocyst formation.

Acknowledgement

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