Free Amino Acid Composition of Some Nitrogen Fixing Blue-Green Algae in Heterocystous and Non-Heterocystous Conditions

Nitrogen fixation in blue-green algae is known only in heterocystous genera. It has also been shown that heterocyst formation in heterocystous blue-green algae is dependent on the amount of combined nitrogen present in the culture medium[1]. The dependence of the formation of heterocyst on the amount of the combined nitrogen present in the culture medium and the observation that only heterocystous genera are known to be nitrogen fixers, indicate the possibility of a close relationship between nitrogen fixation and heterocysts.

Material and method. In the present investigation, amino-acid composition of 4 nitrogen-fixing blue-green algae have been studied in heterocystous and heterocyst-less conditions. The algae, viz., *Nostoc punctiforme* (Kütz.) Hariot, *Scytonema hofmanni* Ag. ex. Born. et Flah, *Scytonema bohneri* Schmidle and *Fischerella muscicola* (Thuret), which were obtained in pure bacteria-free culture, were grown in DE'S[2] culture solution containing 2 g KNO_3/l to obtain algae in non-heterocystous condition, and in nitrogen-free DE's solution for obtaining algae with abundance of heterocysts. The cultures were grown in 500 ml Erlenmeyer Pyrex flasks containing 200 ml of the above-mentioned medium at 28–30 °C under continuous illumination by flourescent tubes.

The alga was harvested after 2 months of growth. The algal mat was separated by centrifugation, washed thoroughly with distilled water, blotted with filter paper and weighed. The extraction of alcohol soluble fraction was prepared after the method of STEWARD et al.[3]. The chromatograms were run in 2 solvents and in 2 directions after the method of CONDENSEN et al.[4]. Comparison of the relative position of the amino-acids with the help of a 'map' prepared by using reference amino-acids, helped in the identification of the amino-acids. A comparison was made of the relative amount of the amino-acid spots using the spot-area method of FISCHER et al.[5]. The area of the spots was measured using an 'Allbrit' planimeter.

Result and discussion. The Table shows the amino-acid composition of 4 species of blue-green algae, viz., *Nostoc punctiforme, Scytonema hofmanni, Scytonema bohneri* and *Fischerella muscicola,* in heterocystous and non-heterocystous conditions produced by growing these on elementary nitrogen and combined nitrogen respectively. It may be noted that the 4 species exhibit both qualitative and quantitative changes in amino-acid composition among themselves and between heterocystous and non-heterocystous stages. Many of the amino-acids recorded in the present study are known to be widely distributed in plants, including various other algae[6–9], fungi and higher plants. γ-aminobutyric acid, which is known to enjoy almost universal distribution in higher plants, has been reported only once by PANDEY and MITRA[9] in heterocystous condition of *Anabaena naviculoides* and its absence in non-heterocystous condition. Out of the 4 species in the present study γ-aminobutyric acid was recorded only in one form, i.e., *Fischerella muscicola,* and its relative quantity was more in non-heterocystous form as compared to heterocystous form.

Some unidentified ninhydrin positive spots (unidentified I–VI) were observed on the paper chromatograms. Whether these are as yet unidentified amino-acids or small peptides is not known. In general the quantity of free amino-acids is less in heterocystous conditions. Whether this decrease in free amino-acid content in the

[1] D. C. PANDEY and A. K. MITRA, Proc. Symp. Algology (ICAR, New Delhi 1960), p. 99.
[2] P. K. DE, Proc. R. Soc., London, B, *127*, 121 (1939).
[3] F. C. STEWARD, R. H. WETMORE, J. F. THOMPSON and J. P. NITSCH, Am. J. Bot. *41*, 123 (1954).
[4] R. CONSDEN, A. H. GORDON and A. J. P. MARTIN, Biochem. J. *38*, 224 (1944).
[5] R. B. FISCHER, D. S. PARRONS and G. A. MORRISON, Nature *161*, 764 (1948).
[6] A. WATANABE, Arch. Biochem. Biophys. *34*, 50 (1951).
[7] A. E. WILLIAMS and R. H. BURRIS, Am. J. Bot. *39*, 340 (1952).
[8] L. FOWDEN, Ann. Bot. *18*, 257 (1954).
[9] D. C. PANDEY and A. K. MITRA, Naturwissenschaften *10*, 248 (1964).

Free amino-acid composition of some blue-green algae in heterocystous and non-heterocystous conditions

	Nostoc punctiforme		*Scytonema hofmanni*		*Scytonema bohneri*		*Fischerella muscicola*	
	Heterocyst	Non-heterocyst	Heterocyst	Non-heterocyst	Heterocyst	Non-heterocyst	Heterocyst	Non-heterocyst
α-Alanine	–	1.8	–	2.5	2.3	3.3	2.7	2.2
Arginine	–	–	–	–	2.2	2.6	2.5	3.9
Aspartic acid	2.0	2.1	2.0	3.1	2.1	2.2	2.2	2.5
Cysteic acid	1.5	–	1.3	1.3	–	1.5	0.9	1.4
Glutamic acid	3.0	3.2	3.3	3.7	2.7	2.7	3.6	–
Glutamine	–	–	–	–	3.1	3.9	3.0	3.5
Glycine and serine	4.2	4.3	2.9	3.8	3.1	3.9	3.0	3.5
Histidine and lysine	–	–	–	–	–	2.5	–	–
Proline	–	–	–	–	–	1.6	–	–
γ-Aminobutyric acid	–	–	–	–	–	–	1.9	3.0
Unidentified (I)	–	2.2	1.8	2.8	1.7	2.9	2.0	2.4
Unidentified (II)	–	–	2.1	3.7	1.8	2.9	2.0	2.5
Unidentified (III)	–	–	2.8	–	–	–	–	2.9
Unidentified (IV)	2.4	–	1.5	3.7	2.3	3.1	1.8	2.1
Unidentified (V)	–	–	1.0	1.3	–	–	–	–
Unidentified (VI)	–	–	–	–	–	–	1.5	1.7

Data expressed as area of the spots in cm^2; –, denotes the absence of the spot.

heterocystous condition, which is obtained by growing these in complete absence of combined nitrogen, is due to the excessive leaching of the amino-acids/peptides to the external medium as reported by others[6,10], or due to the limited synthesis of amino-acid in presence of elementary nitrogen which is enough to support only growth and protein synthesis, or to both interplaying simultaneously, is not known.

That heterocystous stages are accompanied by changes in amino-acid composition has been confirmed. But in different forms the changes are different and in some cases opposite to the other species. It is, therefore, not possible to assign much significance to the qualitative changes which take place in the amino-acid composition with heterocyst formation. This may be probably related to the differences in the intermediary metabolism contributing to nitrogen fixation in different forms.

Zusammenfassung. N_2-fixierende Blaualgen wurden unter Bedingungen gezüchtet, die einerseits zu Heterocysten führten, andererseits jedoch diese Bildung verhinderten. Die Analyse der freien Aminosäuren ergab sowohl qualitative als auch quantitative Unterschiede, ohne dass ein eindeutiger Zusammenhang zwischen der Synthese einzelner Aminosäuren und dem Vorhandensein beziehungsweise der Abwesenheit von Heterocysten beobachtet werden konnte.

V. K. LALORAYA and A. K. MITRA

University of Allahabad, Department of Botany, Allahabad U.P. (India), 22 July 1969.

[10] G. E. FOGG, Proc. Symp. Algology (ICAR, New Delhi 1960), p. 138.

Effect of Methyl Phenyldiazenecarboxylate (Azoester) on the Germination of the Fungus *Trichoderma viride*

Certain stages of cell division apparently depend upon adequate intracellular levels of thiols[1,2]. These thiols are of low molecular weight and probably consist largely of glutathione (GSH). For present purposes, we shall discuss experiments in terms of GSH or thiols of equivalent reactivity.

A new set of reagents has been introduced recently for the intracellular oxidation of GSH to GSSG[3-5]. One of these reagents, methyl phenyldiazenecarboxylate (azoester), reacts with GSH according to the stoichiometry shown in eq. (1).

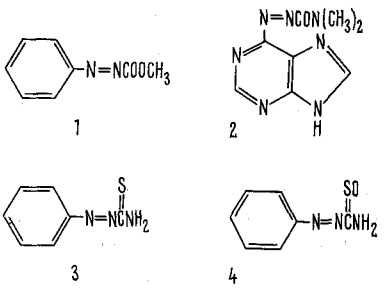
$$2GSH + C_6H_5N{=}NCOOCH_3 \rightarrow GSSG + C_6H_5NHNHCOOCH_3 \quad (1)$$

The complete chemistry of azoester is discussed elsewhere[5]; we must here only point out that a side reaction, hydrolysis, leads to the formation of free radicals which cause intracellular damage. The damage, however, ensues only if the intracellular GSH has been diminished to a very low level.

We have utilized azoester to investigate whether or not GSH was more essential at some stages of germination of the fungus *Trichoderma viride* than at others. RICHMOND and SOMERS[6] have shown that soluble thiols (presumably GSH) increase more rapidly than dry weight or extent of germination in the fungus *Neurospora crassa*.

Spores of *T. viride* were added to minimal media[6,7] to start the germination process. At various times, a $3 \times 10^{-3} M$ solution of azoester was added to the spore suspension in sufficient quantity to make a final azoester concentration of $1 \times 10^{-3} M$. The pH of the medium was between 5 and 6, and the half-life of the azoester was about 30 min under these conditions. Thus, within 1–2 h, the azoester concentration had been reduced to a point where control experiments indicated that it had no effect of any kind. The extent of germination was evaluated by counting under a low power microscope: any spore with an emerging filament was counted as germinated. The results are shown in the Table. Controls are carried out by mixing water with spore suspension and recording the extent of germination at 22 h after initiation. At least 95% of the control spores germinated.

The results indicate that successful germination is prevented or severely delayed by intracellular oxidation of GSH. Further experiments with a thiol-oxidizing agent which does not generate free radicals, 6-purinyldiazenecarboxylic acid N, N-dimethylamid, demonstrated that the destructive action of the free radicals was the



Effect of azoester on germination of fungus *Trichoderma viride*

Time of azoester treatment[a] (h after start)	Extent of germination at + 22 h[b] (% of control)
0	100
4	50
8	3
14	1–2
18	33

[a] Azoester has a half-life of 20 min in neutral aqueous solution.
[b] Time after exposure of spores to minimal media.

[1] D. MAZIA, in *Sulfur in Proteins* (Ed. R. BENESCH; Academic Press, New York 1959), p. 367.
[2] H. STERN, in *Sulfur in Proteins* (Ed. R. BENESCH; Academic Press, New York 1959), p. 391.
[3] N. S. KOSOWER, G. A. VANDERHOFF, E. M. KOSOWER and P-K. C. HUANG, Biochem. Biophys. Res. Commun. *20*, 469 (1965).
[4] N. S. KOSOWER, G. A. VANDERHOFF and I. M. LONDON, Blood *29*, 313 (1967).
[5] N. S. KOSOWER, K. R. SONG and E. M. KOSOWER, Biochem. Biophys. Acta, in press (1969).
[6] D. V. RICHMOND and E. SOMERS, Biochem. biophys. Res. Commun. *25*, 657 (1966).
[7] Minimal medium contains 0.5 g $MgSO_4$, 1.0 g KH_2PO_4, 10 g glucose and 0.5 g DL-alanine in 1000 ml distilled water, pH 5–6.