

SHORT COMMUNICATION

Glutamate Synthase Activity of Heterocysts and Vegetative Cells of the Cyanobacterium *Anabaena variabilis* Kütz.

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Glutamate synthase (GOGAT) activities in cell-free extracts of isolated heterocysts and whole filaments of the cyanobacterium *Anabaena variabilis* Kütz. (ATCC 29413) were determined by measuring [^{14}C]glutamate production from 2-oxo[^{14}C]glutarate or from [^{14}C]glutamine, both of which are GOGAT substrates. There was negligible [^{14}C]glutamate production from 2-oxo[^{14}C]glutarate in the presence of aminooxyacetate, which inhibits aminotransferase activity (<3% of that detectable in whole filament extracts). In experiments using [^{14}C]glutamine, [^{14}C]glutamate production by heterocyst extracts accounted for about 17% of that detected in whole filament extracts, even in the absence of 2-oxoglutarate, ferredoxin and sodium dithionite. It is concluded that virtually all the [^{14}C]glutamate formed from [^{14}C]glutamine in heterocyst extracts is independent of GOGAT activity.

INTRODUCTION

The major route of primary ammonia assimilation in heterocystous cyanobacteria is the glutamine synthetase–glutamate synthase pathway (see Stewart, 1980). Glutamine synthetase (GS) is present both in vegetative cells and in heterocysts, the latter having a higher specific activity (Dharmawardene *et al.*, 1973). In previous studies Thomas *et al.* (1977) reported that very little, if any, glutamate synthase (GOGAT) activity was present in heterocysts of *Anabaena cylindrica* although high activities were found in vegetative cells. Recently, however, Gupta & Carr (1981) have provided evidence that heterocyst extracts show about 13% of the GOGAT activity of vegetative cells. Because of the uncertainty about the GOGAT activity in heterocysts we have re-examined the question using whole filaments and isolated heterocysts of *Anabaena variabilis*.

METHODS

Experimental material. *Anabaena variabilis* Kütz. (ATCC 29413) was grown in BG-11₀ medium (Rippka *et al.*, 1979) at 25 °C and 50 $\mu\text{E m}^{-2} \text{s}^{-1}$ photon flux density. The cells were harvested during the exponential phase and heterocysts were isolated as described before (Hawkesford *et al.*, 1981). Such heterocyst preparations had glutamine synthetase biosynthetic activity (Sampaio *et al.*, 1979) of 74 nmol product formed min^{-1} (mg protein) $^{-1}$ and glucose-6-phosphate dehydrogenase activity [assayed as by Apte *et al.* (1978), except that the assay pH was 7.5] of 48 nmol product formed min^{-1} (mg protein) $^{-1}$.

Enzyme extraction. Cell-free extracts of whole filaments and of isolated heterocysts were prepared in HEPES buffer (50 mmol l^{-1} ; pH 7.5), containing EDTA (5 mmol l^{-1}) and 2-mercaptoethanol (12.5 mmol l^{-1}), by passage through a French pressure cell at 110 MPa. The extract was centrifuged at 7000 g for 10 min at 4 °C and the supernatant was used for enzyme assay after passage through Sephadex G-25.

Glutamate synthase (EC 1.4.7.1) assay. GOGAT activity was determined by measuring the ferredoxin-dependent formation of [^{14}C]glutamate from [^{14}C]glutamine and 2-oxoglutarate. The reaction mixture, in a total volume of 0.7 ml, contained HEPES (50 mmol l^{-1} ; pH 7.5), enzyme extract containing 2 to 3 mg protein, 2-oxoglutarate (5 mmol l^{-1}), glutamine (containing 40 kBq [^{14}C]glutamine) (5 mmol l^{-1}), aminooxyacetate (5 mmol l^{-1}), 0.1 mg

ferredoxin and 1.6 mg Na₂S₂O₄ plus 1.6 mg NaHCO₃. The reaction was initiated by the addition of ferredoxin and dithionite and, after 20 min, terminated by adding 0.7 ml acetone. The reaction mixture was kept anaerobic throughout the assay under an atmosphere of N₂. The reaction mixture was then subjected to descending chromatography on Whatman no. 1 paper using a phenol/water/ammonia (75:21:4, by vol.) solvent system. The glutamate spots, which co-chromatographed with a known standard, were cut out and the radioactivity counted in a toluene-based scintillant using a Packard Tri-carb 2660 scintillation spectrometer. GOGAT activity was also determined by measuring glutamate formation from 2-oxo[5-¹⁴C]glutaric acid and glutamine as previously described (Rai *et al.*, 1981).

Protein measurement. The Lowry method was used with bovine serum albumin as standard.

RESULTS AND DISCUSSION

In a complete reaction mixture containing the cell-free extract, the substrates of GOGAT (2-oxoglutarate and [¹⁴C]glutamine), aminooxyacetate (which inhibits aminotransferase activity; Hopper & Segal, 1962), and ferredoxin and dithionite, which are involved in reductant supply, [¹⁴C]glutamate was formed from [¹⁴C]glutamine both by heterocyst and whole filament extracts (Table 1). The rate of glutamate production in heterocyst extracts in the presence of the complete reaction mixture was approximately 17% of that in whole filament extracts, in accord with the findings of Gupta & Carr (1981), who attributed such glutamate formation in heterocysts, in the absence of aminotransferase activity, to GOGAT activity. However in our heterocyst extracts, the rate of [¹⁴C]glutamate production was similar whether or not 2-oxoglutarate, or ferredoxin and dithionite were added. Such results suggest that glutamate was being produced in the heterocyst extracts by a route other than GOGAT activity or that sufficiently high endogenous concentrations of the various substrates were present. The latter possibility is unlikely to be correct because desalted extracts were used; also a large stimulation in activity was noted when these substrates were added to whole filament extracts. In early studies Haystead *et al.* (1973) reported the presence of glutaminase in cyanobacterial extracts and Lea & Mifflin (1975) reported that in extracts of *Anabaena cylindrica* and *Nostoc ellipsosporum* a considerable amount of glutamate production from glutamine occurred in the presence of aminooxyacetate even when ferredoxin, dithionite or 2-oxoglutarate were omitted from the reaction mixture. Therefore, formation of glutamate in the absence of ferredoxin, dithionite or 2-oxoglutarate is not necessarily attributable to GOGAT activity.

Table 1. *Glutamate production by cell-free extracts of isolated heterocysts and whole filaments of Anabaena variabilis Kütz.*

In the assays using [¹⁴C]glutamine, the basal reaction mixture consisted of HEPES (50 mmol l⁻¹; pH 7.5), aminooxyacetate (5 mmol l⁻¹) and extract. The following compounds were added as indicated: 2-oxoglutarate (5 mmol l⁻¹), glutamine (containing 40 kBq [¹⁴C]glutamine) (5 mmol l⁻¹), 0.1 mg *A. variabilis* ferredoxin and 1.6 mg Na₂S₂O₄ + 1.6 mg NaHCO₃. The final volume was 0.7 ml. In the assays using 2-oxo[¹⁴C]glutarate, the basal reaction mixture and the concentrations of the various constituents were as described above, except that 2-oxo[¹⁴C]glutarate was used instead of [¹⁴C]glutamine.

Additions to basic reaction mixture	Glutamate formation [nmol min ⁻¹ (mg protein) ⁻¹]	
	Heterocyst extract	Whole filament extract
Assays using [¹⁴ C]glutamine		
[¹⁴ C]Glutamine, 2-oxoglutarate, ferredoxin and dithionite	8.7	48.9
[¹⁴ C]Glutamine, ferredoxin and dithionite	7.9	5.4
[¹⁴ C]Glutamine and 2-oxoglutarate	8.3	6.4
Calculated GOGAT activity	0.4-0.8	42.4-42.9
Assays using 2-oxo[¹⁴ C]glutarate		
2-Oxo[¹⁴ C]glutarate, glutamine, ferredoxin and dithionite	1.2	38.6
2-Oxo[¹⁴ C]glutarate, ferredoxin and dithionite	0.00	0.30
2-Oxo[¹⁴ C]glutarate and glutamine	0.07	0.65
Calculated GOGAT activity	1.1	38

To check further for GOGAT activity, we measured the rate of [^{14}C]glutamate production from 2-oxo[^{14}C]glutarate rather than from [^{14}C]glutamine (Table 1). In the absence of added glutamine or reduced ferredoxin there was negligible glutamate formation by the isolated heterocyst extracts and ^{14}C -labelled glutamate production by GOGAT in the heterocyst preparations accounted at the most for 3% of the GOGAT activity of whole filaments (and some or all of this could be due to contamination from vegetative cells or proheterocysts in the heterocyst preparations).

In sum, our data using 2-oxo[^{14}C]glutarate are in accord with those of Thomas *et al.* (1977) who suggested that GOGAT activity is low or undetectable in heterocysts. Our data further suggest that although [^{14}C]glutamate can be produced in heterocyst extracts from added [^{14}C]glutamine, as noted by Gupta & Carr (1981), this is due largely to GOGAT-independent activity.

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