GLUTAMATE SYNTHETASE TYPE ACTIVITY IN HIGHER PLANTS

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1. Introduction

Glutamate dehydrogenase (GDH) is generally regarded as the major route of ammonia incorporation in higher plants [1,2] as well as in microorganisms [3]. Recent studies with procaryotes have, however, shown that in a number of these organisms an alternative ammonium incorporation system is not only present, but may play a key role in the assimilation process, particularly at low or limiting ammonia concentrations. This alternative assimilation system comprises the enzymes glutamine synthetase and glutamine (amide): 2-oxoglutarate amino transferase oxidoreductase (NADP) (glutamate synthetase or GOGAT, see eq. 1) and was first reported by Tempest, Meers and Brown [4] in ammonia limited cultures of *Aerobacter aerogenes* in 1970.

$$\text{Glutamine} + \text{2-oxoglutarate} + \text{NADPH} \rightarrow 2\ \text{glutamate} + \text{NADP}^+ \quad (1)$$

In sycamore (*Acer pseudoplatanus*) cells grown in batch suspension culture with nitrate as the limiting nitrogen source the activity of both NADH and NADPH coupled forms of GDH is low; much lower even than in sycamore cells grown on glutamate. This finding is similar to that for *A. aerogenes* [4] and *Schizosaccharomyces sp.* [5] maintained under similar circumstances where GOGAT activity has been found.

As Young [6] had already reported the presence of glutamine synthetase in cultured sycamore (*Acer Pseudoplatanus*) cells we therefore investigated these same cells for the presence of GOGAT. As a further step, we also looked for the presence of GOGAT in pea (*Pisum sativum*) root tissue.

2. Materials and methods

Suspension cultures of sycamore cells were maintained in batch culture in 250 ml conical flasks as described previously [7]. The nutrient media was as described by Stuart and Street [8] with nitrate as the limiting nitrogen source.

Sycamore cells were harvested over Miracloth and disrupted in a complex medium with a Polytron homogeniser [7]. Cell wall debris was removed by centrifugation at 700 × *g* for 10 min. The resulting supernatant was further centrifuged at 100 000 × *g* for 30 min, providing supernatant and particulate fractions which were immediately assayed for enzymic activity.

Pea roots were grown under sterile conditions [8] in the absence of an exogenous nitrogen source. After 5 days they were harvested and the intact seedling root incubated in a solution of 10mM potassium nitrate for 4 hr. The roots were then harvested and homogenised in 40mM glycylglycine pH 7.2. The extracts were then centrifuged and fractions prepared as described for the sycamore cells.

Protein in the enzyme extracts was determined by the Folin method.

Assays for glutamate dehydrogenase and glutamine synthetase were carried out as described previously [6, 9]. The assay system for GOGAT was fundamentally as described by Tempest et al. [10] with certain modifications described later.

Reaction rates were measured at 340 nm and 25°C using a Gilford recording spectrophotometer; rates were linear for at least 5 min.

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3. Results and discussion

3.1. *Presence of GOGAT in sycamore cells and in pea roots*

NADPH oxidation in the presence of L-glutamine and 2-oxoglutarate was observed in the three stages of sycamore cell growth monitored (table 1) and the reaction rate was found to be linear with volume of enzyme extract. In all cases GOGAT activity was much higher than GDH activity, and glutamine synthetase had an activity roughly 10 times that of GOGAT. Roughly one third of the GOGAT activity was associated with the particulate fraction.

GOGAT and glutamine synthetase activities were also found in the extract of pea root tissue and the activities of both enzymes exceeded the activities of glutamate dehydrogenase (table 2).

Table 1

Activities of enzymes of ammonia assimilation in cultured sycamore cells, with NO^-_3 as limiting nitrogen source

Age of cells (days)	Fraction	GDH (NADP)	GDH (NAD)	Glutamine synthetase	GOGAT
		(nmol per min per mg protein)			
5	S	2.2	2.4	1100	11.84
	P	0	4.9	0	7.40
11	S	2.1	7.1	1876	175.4
	P	0	3.5	0	63.6
14	S	2.4	11.0	1383	174.8
	P	0	N.M.	0	87.4

GOGAT assay mixture contained NADPH (0.4 mM), glutamine (5 mM), oxoglutarate (10 mM), glycylglycine (40 mM) pH 7.6. Corrections were made for NADPH oxidase activity in the extract. Protein was measured by the Folin method. S = 100 000 *g* supernatant; P = 100 000 particulate fraction; N.M. = not measured.

Table 2

Activities of enzymes of ammonia assimilation in pea roots incubated in 10 mM potassium nitrate for 4 hr

GDH (NAD)	Glutamine Synthetase	GOGAT
	(nmol. per min per mg. protein)	
21.2	24.4	139.9

Assays were carried out on the 100 000 *g* supernatant and were as described in table 1.

Table 3

Rates of NADPH oxidation in extracts of sycamore cells in the presence of glutamine or asparagine and 2-oxoglutarate or oxaloacetate

Acid acceptor	Amide donor	
	Glutamine	Asparagine
	(nmoles NADPH utilised/min/ml extract)	
Oxoglutarate	173.6	493.8
Oxaloacetate	69.4	227.6

The levels of glutamine and oxoglutarate in the reaction mixture were as described in table 1. Asparagine was present at 5 mM and oxaloacetate at 10 mM.

3.2. *Characteristics of the reaction*

Since GOGAT activity greatly exceeded GDH activity (table 1) the oxidation of NADPH could not be due to a coupled glutaminase-GDH system. In support of this there was no appreciable accumulation of ammonia during the reaction, and addition of ammonia to the reaction system did not increase NADPH oxidation. However some oxidation of NADPH in the GOGAT assay may be contributed by these other enzymes, but the extent cannot exceed the activity of GDH.

In contrast to the situation in *A. aerogenes* [4] and *Schizosaccharomyces* [5] where the enzyme is specific for glutamine as the amide donor, the GOGAT system from sycamore cells was found to use either glutamine or asparagine (table 3). At the same time the plant system was able to utilise oxaloacetate or 2-oxoglutarate as the acceptor with either amide donor (table 3). However, in common with the reaction system from microorganisms the plant enzyme showed no significant activity with pyruvate.

The plant GOGAT reaction was active with NADPH and NADH, showing a slightly higher activity with NADPH (fig. 1). This is in contrast to microorganisms where the reduced pyridine nucleotide specificity has been shown to vary between different species [4,5,10].

Apparent k_m values determined on an unpurified enzyme fraction were 5.2×10^{-4} M for glutamate and 1.2×10^{-5} M for asparagine with the concentration of NADPH at 0.4mM and 2-oxoglutarate at 10 mM.

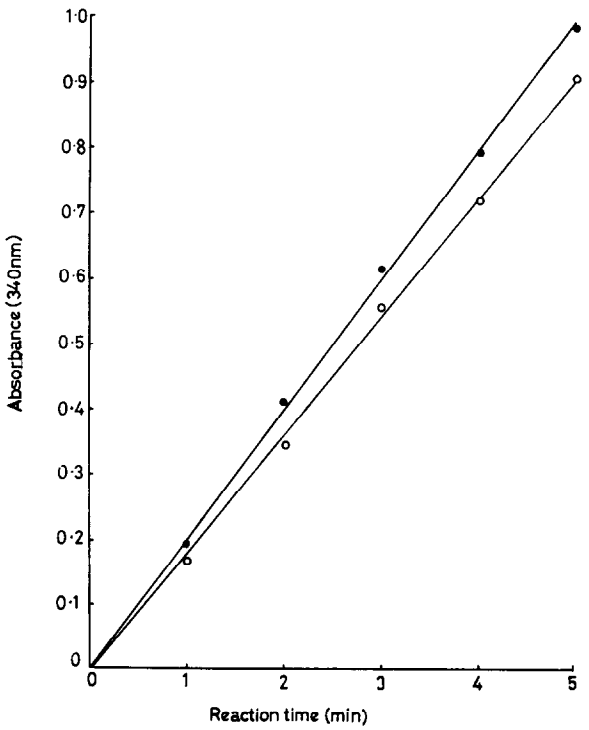

Fig. 1. Utilisation of either NADPH (●——●) or NADH (○——○) as hydrogen donor in the GOGAT reaction by extracts of sycamore cells.

3.3. *Discussion*

The findings suggest the presence of a GOGAT-like reaction system in both sycamore and pea root cells in limiting nitrate (ammonia) conditions. It is impossible from the present data to determine whether the enzyme activity in these higher plant tissues is exactly similar to that observed in microorganisms. Marked contrasts with the microbial reaction system come from the versatility of the plant system in the use of either glutamine or asparagine as an amide donor, and 2-oxoglutarate or oxaloacetate as an acceptor. If the plant reaction is catalysed by only one enzyme, which has a broad specificity for donor and acceptor, then the enzyme may be somewhat different from the microbial one, although presumably having the same general reaction mechanism. The occurrence of substantial glutamine synthetase and GOGAT activity in higher plant cells grown under limiting nitrogen conditions suggests that these two reactions may form an important alternative assimilation pathway to glutamate dehydrogenase, an enzyme which has a low activity in many plant cells and also which, in general, has a high k_m value for ammonia [11]. Such a situation could have important implications in our understanding of nitrogen assimilation in plant tissues and may offer a possible explanation of how some plants are able to flourish without symbiotic association on nitrogen limited soils, such as those plants growing in acid moorland conditions.

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