Properties and Physiological Functions of Glutamate Dehydrogenase and Glutamate Synthase in Ammonium-Assimilating Metabolism in Euglena gracilis Z

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Properties and Physiological Functions of Glutamate Dehydrogenase and Glutamate Synthase in Ammonium-Assimilating Metabolism in *Euglena gracilis Z*

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Abstract

NADP-dependent glutamate dehyrogenase (GDH; EC 1.4.1.4) and NADH-dependent glutamate synthase (GOGAT; EC 1.4.1.14), the key enzymes in ammoina assimilating reactions in *Euglena gracilis* were studied. Repression of the synthesis of GDH during growth of *E. gracilis* on L-glutamate or Casamino acids was completely removed by the addition of ammonia and glucose. By contrast, the addition of L-glutamate or Casamino acids to the cells growing ammonium and glucose resulted in a strong repression of GDH. The activity of GOGAT was constant upon either addition.

Some enzymological properties of the two enzymes were also studied.

From these results, we concluded that GDH serves mainly as an anabolic agent in the ammonia assimilation while GOGAT plays an auxilliary role of ammonia assimilation in this organism.

Introduction

In many organisms, assimilation of inorganic nitrogen into organic forms (amino acids) proceeds primarily by the action of glutamate dehydrogenase (GDH; EC 1.4.1.4) or through coupled reactions involving glutamine synthetase (GS; EC 6.3.1.2) and glutamate synthase (GOGAT; EC 1.4.1.14)[1].

However, the enzymes involved in the assimilation of ammonia in *Euglena* have not been studied in detail. Tokunaga *et al.* reported the presence of NADP-dependent GDH[2] and Miyatake and Kitaoka found occurrence of NADH-dependent GOGAT in this organism[3]. The relation of the physiological role of these enzymes in the nitrogen metabolism in *Euglena* has not been elucidated.

In the present paper, we report on variations of the activity of these enzymes upon different nutrients and on their kinetic properties, and suggest difference in the physiological role of the two enzymes in the assimilation of ammonia in *Euglena.*

Material and Methods

Chemicals. All chemicals were analytical grade reagents manufuctured in Japan.

Culture. A streptomycin-bleached mutant of *E. gracilis* was cultured in the Oda's medium[4]. Cultivation was conducted under illumination (3000 lux) at 27°C with shaking (90 strokes/min). Depending on the purpose of the experiment, the nitrogen compounds in the medium were replaced by glutamate, Casamino acids or $(NH_4)_2HPO_4$. Growth was measured by counting the cell number with a hemocytometer. Protein was determined by the method of Lowry *et al.* using bovine albumin as a standard[5].

Preparation of cell extract. Harvested E. gracilis cells were washed in a 20 mM glycyl-glycine buffer, pH 7.4, containing 0.25 M sucrose and disrupted in the same buffer

by sonication (10 Kc) for 30 sec. The supernatant obtained by centrifugation of the sonicate at 10,000 × *g* for 10 min was used as a crude enzyme. Disruption of the cells by the digestion technique and fractionation of cell homogenate by differential centrifugation were performed by the methods of Tokunaga *et al.*[2] and Shigeoka *et al.*[6]

Enzyme assays. NADP-dependent succinic semialdehyde dehydrogenase (EC 1.2.1.16) was assayed according to Tokunaga *et al.*[7] and other marker enzymes were assayed as described by Yokota and Kitaoka[8]. *Euglena* GDH and GOGAT were assayed by the methods described in the previous paper[3].

Results and Discussion

Effect of culture conditions on the levels of the GDH and GOGAT activities in Euglena.

The specific activities of GDH and GOGAT in crude extracts of *E. gracilis* grown on various carbon and nitrogen sources are shown in Table 1. Nearly same levels of the

Table 1. Specific activities of GDH and GOGAT in extracts from early stationary phase cells (5 days after inoculation) of *E. gracilis* when grown on various carbon and nitrogen sources

Carbon sources	Nitrogen sources	Specific activity of GDH and GOGAT (nmole/mg protein/min)	
55 mM glucose	10 mM $(NH_4)_2HPO_4$	391.7	60.7
75 mM malate	10 mM $(NH_4)_2HPO_4$	386.8	55.1
55 mM glucose + 34 mM glutamate	34 mM glutamate	3.2	58.9
55 mM glucose + 0.3 % Casamino acids	0.3 % Casamino acids	1.5	60.1

The supernatant obtained by centrifugation of the sonicate at 10,000 × *g* for 10 min was used as a crude enzyme. Results are given in average values from three determinations.

specific activity of GDH were found on glucose, a glycolytic substrate or on malate, a gluconeogenetic substrate, when ammonium was used as the sole source of nitrogen. However, extracts from the cells grown in glutamate or Casamino acids as the sole nitrogen source contained only very low level of GDH activity.

By contrast, the GOGAT activities in the cells grown on various carbon and nitrogen sources were nearly identical. These results suggest that the level of GDH activity is strongly regulated in *E. gracilis* and that this enzyme functions mainly to catalyze the glutamate synthesis while GOGAT has a different function.

Regulation of the levels of GDH and GOGAT activities in growing Euglena cells.

The specific activity of GDH in the extract of *E. gracilis* grown on glutamate was very low (Fig. 1) but the addition of both $(NH_4)_2HPO_4$ and glucose to the culture medium rapidly increased the specific activity of GDH.

On the other hand, the addition of glutamate to the culture with $(NH_4)_2HPO_4$ as the nitrogen source produced a dramatic decrease of the GDH activity, suggesting that

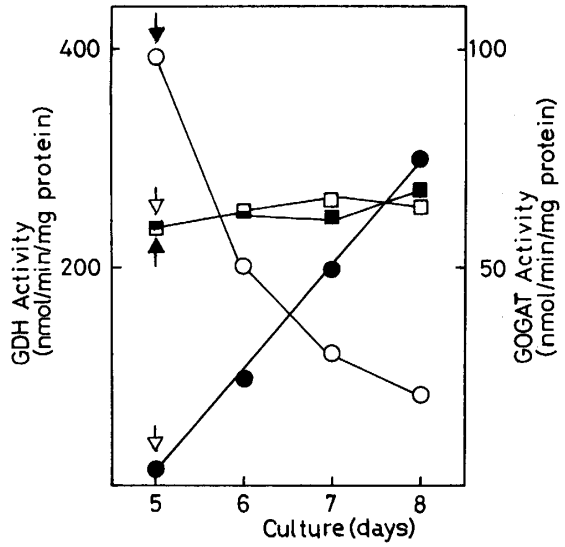

Fig. 1 Effect of addition of glutamate or ammonium ion on the profiles of GDH and GOGAT activities during growth of *E. gracilis*.
Symbols: ● ; GDH activity of cultured in glutamate, ○ ; GDH activity of cultured in $(NH_4)_2HPO_4$, ■ ; GOGAT activity of cultured in glutamate, □ ; GOGAT activity of cultured in $(NH_4)_2HPO_4$, ↓ ; addition of 34 mM glutamate, ⇩ ; addition of 10 mM $(NH_4)_2HPO_4$.

glutamate represes strongly the synthesis of GDH. The addition of $(NH_4)_2HPO_4$ and glucose to the glutamate culture, as well as the addtion of glutamate and glucose to the $(NH_4)_2HPO_4$ culture, increased only slightly the GOGAT activity.

Tompest *et al.*[9] have shown that when ammonia is limiting to the growth of *Aerobacter aerogenes*, the level of GOGAT rises while that of GDH falls. By contrast, at high levels of ammonia, with limited carbon sources, the GDH level rises while the GOGAT level falls. In *Euglena*, informations on such relations have not been available.

Two types of GDH have been demonstrated to exist in yeast[10] and several fungi[11]. The NADP-dependent GDH (EC 1.4.1.4) appears to have an anabolic function (glutamate synthesis) and synthesis of this enzyme is repressed by glutamate[11]. The NAD-dependent GDH (EC 1.4.1.2), on the other hand, appears to serve in the catabolic function (oxidative deamination of glutamate) and is induced by glutamate[11]. The concurrent regulation of these isozymes may possible have a significant role during differentiation of *Schizophyllum*[11] and Neurospora[12]. Most bacteria, however, are thought to contain only one type of GDH, an anabolic, NADP-dependent one[13].

Ferredoxin-dependent GOGAT present in chloroplasts in higher plants and green algae, is involved together with GS in the incorporation of ammonia into the α-amino group of amino acids[1]. We have reported that *E. gracilis* contains only NADP-dependent GDH[2] and that the GOGAT is NADH-specific and localized in cytosol but not in chloroplasts[3]. These results indicate that *Euglena* GOGAT, is independent of the ferredoxin-type GOGAT in chloroplasts and has a different physiological function.

Kinetic properties of GDH and GOGAT.

The apparent kinetic constants of crude GDH and GOGAT were determined. Reciplocal plots of the saturation curves for substrates are linear and obey the Michaelis-Menten equation. The results are summarized in Table 2, in which properties and location of the two enzymes are also described.

The *Km* values for the substrates in glutamate synthesis were relatively much smaller

Table 2. Some properties of GDH and GOGAT of *Euglena gracilis*

Properties		GDH	GOGAT*
Km	values for substrates		
	L-glutamate	6.7×10^{-4} M	
	$NADP^+$	1.0×10^{-4} M	
	NH_4^+	4.9×10^{-4} M	
	NADPH	3.2×10^{-5} M	
	α-ketoglutarate	2.5×10^{-4} M	1.5×10^{-5} M
	L-glutamine		3.0×10^{-4} M
	NADH		2.0×10^{-5} M
Optimum pH		9.5 (ammonia formation)	7.5
		8.0 (glutamate formation)	
Optimum temperature		35°C	30°C
Sigmoidal behavior		ND	ND
Localization		cytosol	cytosol

* The results are from the work of Miyatake and Kitaoka[3].

than the *Km* for the reverse reaction. Furthermore, the V_{max} values for the glutamate synthesis were 2-fold higher than those of glutamate degradation by GDH. The reported *Km* values of GDH for ammonia in most organisms are about 4 mM, indicating a low affinity of the enzyme for ammonia.[1] Some unicellular photosynthetic organisms have GDH with suitable characteristics for ammonia assimilation; the *Km* for ammonia of *Chlorella* GDH is 3-5 $\times$ 10^{-4} M[1] and that of *Euglena* enzyme is shown to be 4.9×10^{-4} M.

Euglena GOGAT requires specifically NADH as an electron donor. NADPH and ferredoxin do not replace NADH[3]. This cofactor specificity is similar to that of bacterial[14] and non-chlorophyllous plant enzymes[15] and is completely different from the specificity of chloroplast enzymes[1].

Table 2 also shows that GDH and GOGAT are localized in cytosol but they are virtually absent in mitochondrial and chloroplast fractions. These results suggest that the assimilation of inorganic nitrogen into organic forms proceeds in cytosol by the action of these two enzymes. The GDH activity for glutamate synthesis, however, was 6-7 times higher than that of GOGAT under physiological conditions of *Euglena* cytosol, pH 7.4 and 27°C.

Kempner and Miller showed that *E. gracilis* utilizes glutamate best among many amino acids[16]. Oda *et al.*[4] revealed that glutamate transport in *Euglena* follows normal Michaelis-Menten kinetics and the *Km* value of transport system (s) is 30 μM. Extracts from the cells grown on glutamate or Casamino acids showed a very low level of GDH activity while GOGAT levels were kept constant. This result suggests that the incorporated glutamate strongly regulates the level of GDH activity and that the incorporated glutamate is metabolized directly by strong transaminases[17].

We have recently reported occurrence of two type of isocitrate dehydrogenases in *E. gracilis;* one is NAD-linked (EC 1.1.1.41) and located in mitochondria and the other is NADP-linked (EC 1.1.1.42) and located in mitochondria and cytosol fractions[17]. Eighty percent of NADP-dependent enzyme is located in cytosol and this suggests that this enzyme supply NADPH and α-ketoglutarate that are substrates for NADP-dependent

GDH. In other words, a cycle system of NADPH between GDH and isocitrate dehydrogenase in this protozoon is actually operative.

These results have led us to the conclusion that the ammonia assimilation is mainly catalyzed by NADP-dependent GDH and that NADH-linked GOGAT is auxilliay in function to GDH in *Euglena*.

References

1. MIFLIN, B. J., and LEA, P. J. (1976). The Pathway of Nitrogen Assimilation in Plants. *Phytochem.*, **15**, 873.
2. TOKUNAGA, M., NAKANO, Y. and KITAOKA, S. (1979). Subcellular Localization of the GABA-Shunt Enzymes in *Euglena gracilis* Strain Z. *J. Protozool.*, **26**, 471.
3. MIYATAKE, K. and KITAOKA, S. (1981). NADH-Dependent Glutamate Synthase in *Euglena gracilis* z. *Agric. Biol. Chem.*, **45**, 1727.
4. ODA, Y., NAKANO, Y. and KITAOKA, S. (1982). Utilization and Toxicity of Exogeneous Amino Acids in *Euglena gracilis*. *J. Gen. Microbiol.*, **128**, 853.
5. LOWRY, O. H., ROSEBROUGH, N. J., FARR, A. L. and RANDAL, R. J. (1951). Protein measurement with Folin phenol reagent. *J. Biol. Chem.*, **193**, 265.
6. SHIGEOKA, S., NAKANO, Y. and KITAOKA, S. (1979). Some Properties and Subcellular Localization of L-Gulono-γ-lactone Dehydrogenase in *Euglena gracilis* Z. *Agric. Biol. Chem.*, **43**, 2187.
7. TOKUNAGA, M., NAKANO, Y. and KITAOKA, S. (1976). Separation and Properties of the NAD-linked and NADP-linked Isozymes of Succinic Semialdehyde Dehydrogenase in *Euglena gracilis*. *Biochim. Biophys. Acta* **429**, 55.
8. YOKOTA, A. and KITAOKA, S. (1979). Occurrence and Operation of Glycollate-Glyoxylate Shuttle in Mitochondria of *Euglena gracilis* Z. *Biochem. J.*, **184**, 189.
9. TEMPEST, D. D., MEERS, J. L. and BROWN, C. M. (1970). Synthesis of Glutamate in *Aerobacter aerogenes* by a hitherto unknown route. *Biochem. J.*, **115**, 405.
10. HIEHOLZER, G. and HOLZER, H. (1963). Repression der synthese von DPN-abhangiger glutaminsäuredehydrogenase in *Saccharomyces cerevisiae* durch ammoniumionen. *Biochem. Z.*, **333**, 175.
11. DENNEN, D. W. and NIDERPRUEM, D. J. (1967). Regulation of glutamate dehydrogenases duing morphogenesis of *Shizophyllum commune*. *J. Bacteriol.*, **93**, 904.
12. SNAWAL, B. D. and LATA, M. (1962). Concurrent regulation of glutamic dehydrogenases of *Neurospora*. *Arch. Biochem. Biophys.*, **97**, 582.
13. VARRICCHIO, F. (1969). Control of glutamate dehydrogenase synthesis in *Escherichia coli*. *Biochim. Biophys. Acta* **177**, 560.
14. NAGATANI, H., SHIMIZU, M. and VALENTINE, R. C. (1971). The mechanism of ammonia assimilation in nitrogen fixing bacteria. *Arch. Mikrobiol.*, **29**, 164.
15. BEEVERS, L. and STOREY, R. (1976). Glutamate synthetase in developing cotyledons of *Pisum sativum*. *Plant Physiol.*, **57**, 862.
16. KEMPNER, E. S. and MILLER, J. H. (1965). The molecular biology of *Euglena gracilis*. I. Growth conditions and cellular composition. *Biochim. Biophys. Acta* **104**, 11.
17. ODA, Y., MIYATAKE, K., NAKANO, Y. and KITAOKA, S. (1981). Subcellular Location and Some Properties of Isocitrate Dehydrogenase Isozymes in *Euglena gracilis*. *Agric. Biol. Chem.*, **45**, 2619.