Glutamine Degradation Through the ω -Amidase Pathway in *Saccharomyces cerevisiae*

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A glutamine transaminase activity has been identified in *Saccharomyces cerevisiae*, and the existence of the ω -amidase activity previously described in this yeast has been confirmed. The glutamine transaminase utilizes different 2-oxo acids as substrates, including pyruvate and glyoxylate, and is regulated by the available nitrogen source. The glutamine transaminase activity decreases when lysine or glycine is added to the medium; the inhibition by lysine diminishes under microaerophilic culture conditions.

INTRODUCTION

Glutamine plays a central role in nitrogen metabolism in micro-organisms (see the preceding paper: Soberón & González, 1987). We have studied the role of glutaminases in glutamine assimilation in *Saccharomyces cerevisiae* and found that there are at least two glutaminases which have different pH optima (Soberón & González, 1987). One of them (glutaminase A) is membrane bound, while the other (glutaminase B) is cytoplasmic. Glutaminase B is negatively modulated by some 2-oxo acids.

The ω -amidase pathway constitutes an alternative route for glutamine assimilation (Cooper & Meister, 1977). In this pathway, glutamine is transaminated to yield different amino acids and 2-oxoglutaramate through the participation of a glutamine transaminase; subsequently, the 2-oxoglutaramate is hydrolysed, giving rise to 2-oxoglutarate and ammonium by the action of an ω -amidase. Evidence for the degradation of glutamine by the ω -amidase pathway in mammalian systems is well documented (Cooper & Meister, 1981). The operation of this metabolic route has also been studied in *Neurospora crassa* (Calderón *et al.*, 1985), and in *S. cerevisiae* the activity of ω -amidase has been determined (Cooper & Meister, 1977). Since glutaminase B is negatively modulated by 2-oxo acids (Soberón & González, 1987) that can be regarded as substrates of the glutamine transaminase, we examined whether the enzymes of the ω -amidase pathway were present in *S. cerevisiae*.

METHODS

S. cerevisiae strains. The wild-type strain S288C (*MAT α ; mal1 ; gal2*) came from the Cold Spring Harbor Laboratory, New York, USA. The selection and partial characterization of mutant CN10 were described by Soberón & González (1987).

Growth conditions. Strains were routinely grown on minimal media (MM) containing salts, trace elements and vitamins following the formula of the Difco Yeast Nitrogen Base plus 1% (w/v) amino acid as required. Glucose (2%, w/v) was used as carbon source; 40 mM-(NH₄)₂SO₄, 0.1% (w/v) glutamine or 0.1% (w/v) glutamate were used as nitrogen source. Rich medium (YPD) contained 2% (w/v) glucose, 1% (w/v) yeast extract, 2% (w/v) peptone and 2% (w/v) agar when needed. Cells were incubated at 30 °C with agitation (175 r.p.m.) unless otherwise stated. Growth was followed by measuring optical density at 650 nm.

Abbreviations: MM, minimal medium; YPD, rich medium.

Fig. 1. Growth of the wild-type strain S288C (●) and mutant CN10 (○) on glutamine as nitrogen source: (a) with agitation at 175 r.p.m., volume of medium/flask capacity = 1/6; (b) statically.

Glutamine transaminase activity. Soluble extracts were prepared by grinding whole cells in 50 mM-sodium pyrophosphate buffer (pH 8.5), using a Braun homogenizer and glass beads. The extracts were centrifuged for 20 min at 9000 g, and the supernatant was desalted on a Sephadex G25 column equilibrated and eluted with the extraction buffer. The activity was assayed by measuring 2-oxoglutarate production, phenylalanine production or phenylpyruvate disappearance (Calderón *et al.*, 1985; Cooper, 1978; Cooper *et al.*, 1980). Units of activity are nmol 2-oxoglutarate or phenylalanine produced min^{-1} , or nmol phenylpyruvate consumed min^{-1} .

Glutamine transaminase- ω -amidase coupled reaction. Soluble extracts were prepared as described for glutamine transaminase activity. The activity was assayed as reported for 2-oxoglutarate production and the reaction was stopped with 17% (w/v) trichloroacetic acid. The reaction mixture was neutralized and the amount of NH_4^+ produced was estimated with beef glutamate dehydrogenase by following NADH oxidation at 340 nm (White, 1970).

Protein determination. This was done by the Lowry method, using bovine serum albumin as standard.

Chemicals. All amino acids, Sephadex G25, 2-oxo acids and bovine serum albumin were obtained from Sigma.

RESULTS

Phenotypic characterization of a mutant impaired in glutamine utilization under microaerophilic conditions

In the preceding paper we described a mutant (CN10) with an alteration in glutaminase B activity that results in *in vivo* inactivation of this enzyme. The mutant grows poorly on glutamine as sole nitrogen source (Fig. 1a). Glutaminase B from the wild-type strain is inhibited by pyruvate; this 2-oxo acid accumulates when this strain is grown under microaerophilic conditions, thus resulting in *in vivo* inactivation of the enzyme (Soberón & González, 1987). Since the wild-type strain is able to grow under microaerophilic conditions with glutamine as sole nitrogen source (Fig. 1b), we determined whether mutant CN10 could grow under these conditions. The mutant had the same doubling time as the wild-type with glutamine as sole nitrogen source in static cultures (Fig. 1b). Since under this condition, glutaminase B is inhibited by the accumulation of pyruvate (Soberón & González, 1987), we decided to determine whether the enzymes of the ω -amidase pathway were present in *S. cerevisiae*, since these could provide an alternative pathway for glutamine degradation. The first enzyme of this pathway, glutamine transaminase, utilizes various 2-oxo acids as substrates (Cooper & Meister, 1977), suggesting that the degradation of glutamine through glutaminase B or glutamine transaminase could be determined by the pool of 2-oxo acids present.

Table 1. *Glutamine transaminase activity in strain S288C*

Cells were grown for 12 h on MM with $(\text{NH}_4)_2\text{SO}_4$ as nitrogen source. Assay conditions: phenylpyruvate 3 mM, glutamine 10 mM, 6-diazo-5-oxo-L-norleucine (DON) 3 mM, oxamic acid 10 mM.

Assay condition	Glutamine transaminase activity [U (mg protein) ⁻¹]		
	2-Oxoglutarate	Phenylpyruvate	Phenylalanine
Complete system	5.3	5.3	5.6
Without glutamine	ND	0.73	—
Without phenylpyruvate	ND	—	—
Without DON	2.4	—	—
Without oxamic acid	ND	ND	—

ND, Not detected; —, not determined.

Table 2. *Glutamine transaminase activity in strain S288C, with different 2-oxo acids as substrate*

Cells were grown for 12 h on MM with $(\text{NH}_4)_2\text{SO}_4$ as nitrogen source. Assay conditions: glutamine 20 mM and 2-oxo acid as stated. Activity was assayed by following 2-oxoglutarate production.

2-Oxo acid	Glutamine transaminase activity [U (mg protein) ⁻¹]
Glyoxylate (5 mM)	11.0
Pyruvate (5 mM)	3.2
Phenylpyruvate (3 mM)	3.0
2-Oxosuccinamate (5 mM)	3.5
2-Oxadipate (5 mM)	1.4
2-Oxoglutarate (5 mM)	ND

ND, Not detected.

Determination of the enzymes involved in the ω -amidase pathway

Glutamine transaminase activity was present in *S. cerevisiae* strain S288C (Table 1). The specific activity was the same, regardless of the method used to determine it. The reaction was inhibited by oxamic acid, which is a specific inhibitor of transaminases. 6-Diazo-5-oxo-L-norleucine (DON) was needed in the assay mixture; this is an inhibitor of the ω -amidase activity which is necessary to allow optimal accumulation of 2-oxoglutarate in *N. crassa* (Calderón *et al.*, 1985). We also determined the coupled activity of the glutamine transaminase and the ω -amidase, measuring the phenylpyruvate-dependent release of ammonium from glutamine. This activity corresponded to about 70% of the activity found for the glutamine transaminase.

The glutamine transaminase was assayed with different 2-oxoacids as substrates (Table 2): the highest activity was found with glyoxylate. It is worth noting that this enzyme could utilize pyruvate and glyoxylate as substrates, both of which are inhibitors of glutaminase B (Soberón & González, 1987). 2-Oxoglutarate was a very poor substrate, even though it is also an inhibitor of glutaminase B (Soberón & González, 1987). These results confirm the existence of the ω -amidase pathway in *S. cerevisiae* (Cooper & Meister, 1977).

Regulation of glutamine transaminase

In order to analyse the participation of the ω -amidase pathway in the degradation of glutamine and in the synthesis of some amino acids, glutamine transaminase activity was assayed in the wild-type strain after 12 h growth on different nitrogen sources (Table 3). Activity was high after incubation on glutamate, glutamate plus ammonium, glutamine, and glutamine

Table 3. *Glutamine transaminase activity in strain S288C grown on different nitrogen sources*

Cells were grown for 12 h on MM with different nitrogen sources. Assay conditions: phenylpyruvate 3 mM, glutamine 10 mM. Activity was assayed by following 2-oxoglutaramate production. Culture conditions: volume of medium/flask capacity = 1/2.

Nitrogen source	Glutamine transaminase activity	
	[U (mg protein) ⁻¹]	
(NH ₄) ₂ SO ₄ + glutamate	10.81	(100%)
Glutamate	10.39	(96.1%)
(NH ₄) ₂ SO ₄ + glutamine	8.7	(80.5%)
Glutamine	8.24	(76.2%)
(NH ₄) ₂ SO ₄	5.1	(47.17%)
YPD (rich medium)	2.02	(18.6%)
(NH ₄) ₂ SO ₄ + lysine	1.29	(11.9%)
(NH ₄) ₂ SO ₄ + glycine	1.69	(15.7%)
(NH ₄) ₂ SO ₄ + alanine	4.5	(42.2%)
(NH ₄) ₂ SO ₄ + aspartate	7.9	(73.5%)

Table 4. *Glutamine transaminase activity in strain S288C and mutant CN10 grown statically (microaerophilic conditions) or with agitation*

Cells were grown for 12 h on MM with 0.1% of glutamine or glutamine plus lysine. Assay conditions: phenylpyruvate 3 mM, glutamine 10 mM. Glutamine transaminase activity was assayed by following 2-oxoglutaramate production and is expressed as U (mg protein)⁻¹; the percentage inhibition is shown in parentheses.

Strain	Agitated*		Static	
	Gln	Gln + Lys	Gln	Gln + Lys
S288C	8.24	1.13 (84%)	8.37	4.6 (45%)
CN10	6.30	1.00 (84%)	8.4	7.6 (9.5%)

* At 175 r.p.m.; volume of medium/flask capacity = 1/2.

plus ammonium, but was low in rich medium (YPD) and in MM supplemented with lysine or glycine. The inhibitory effect of lysine and glycine could reflect the participation of glutamine transaminase in the synthesis of these amino acids, since aspartate, which is synthesized by transamination with glutamate (de Robichon-Szulmajster *et al.*, 1966) did not decrease the glutamine transaminase activity (Table 3). In these experiments the inhibition of glutamine transaminase by lysine was determined in agitated cultures, in which the degradation of glutamine apparently occurs through glutaminase B (Soberón & González, 1987). We also analysed this effect under different conditions of agitation (Table 4). The degree of inhibition by lysine was much greater in the agitated cultures than in the static cultures. Mutant CN10 showed similar behaviour; when this strain was grown under microaerophilic conditions, the transaminase activity showed only 10% inhibition by lysine (Table 4).

DISCUSSION

The data presented show that glutamine transaminase and ω -amidase, the two enzymes that constitute the ω -amidase pathway, are present in *S. cerevisiae*. Glutamine transaminase activity was high in extracts obtained from cells grown with glutamine or glutamate, conditions in which the intracellular glutamine pool is expected to be high (Dubois & Gresson, 1974). Glutamine transaminase activity was increased in the presence of glutamine and decreased in the presence of lysine or glycine, suggesting that the latter amino acids could be synthesized through the action of this transaminase, and that glutamine positively modulates its activity.

Fig. 2. Schematic diagram of the proposed operation of the ω -amidase pathway and glutaminase B. The model proposes that glutamine can be degraded through two different pathways, depending on the intracellular concentration of pyruvate, which is an inhibitor of glutaminase B activity and a substrate of glutamine transaminase.

2-Oxoadipate and glyoxylate can transaminate with other amino acids (Matsuda & Ogur, 1969; Ulane & Ogur, 1972). To find which amino acids are synthesized through the action of glutamine transaminase requires the isolation of mutants devoid of this activity. In the preceding paper we showed that glutaminase B is inhibited by pyruvate and glyoxylate, both of which are substrates of the glutamine transaminase (Soberón & González, 1987). These data suggest that the intracellular pool of these 2-oxo acids could determine which of the two degradative pathways would operate in a given physiological condition. Furthermore, we have shown that 2-oxoglutarate inhibits the glutaminase, even though it is not a substrate of the transaminase (Soberón & González, 1987). However, 2-oxoglutarate is also utilized by glutamate synthase, which can be regarded as an enzyme that also participates in glutamine catabolism (Roon *et al.*, 1974). We are studying the regulation and role of glutamate synthase in glutamine utilization, in order to establish if there is coordination of the activities of these three enzymes.

We have shown that the mutant CN10, which is altered in glutaminase B, is able to grow with glutamine as sole nitrogen source under microaerophilic conditions but not in agitated cultures, and that in microaerophilic conditions pyruvate accumulates and glutaminase activity decreases (Soberón & González, 1987). Furthermore, the activity of glutamine transaminase measured in extracts obtained from cells grown in microaerophilic conditions is only partially inhibited by lysine, suggesting that under this condition the regulation of this enzyme is different from that found in extracts obtained from cells grown in agitated cultures. All these data suggest that the operation of the glutaminase and the ω -amidase pathways is coordinately regulated. That is, under conditions in which pyruvate accumulates, the glutaminase is inhibited and glutamine is degraded through the glutamine transaminase. In conditions in which accumulation of pyruvate is decreased (agitation), the enzyme participating in the catabolism of glutamine would be the glutaminase.

A diagrammatic representation of the model presented above is shown in Fig. 2. The isolation of mutants altered in glutamine transaminase is important, since this could be a way to demonstrate the operation of the ω -amidase pathway, to study which amino acids are synthesized through glutamine transaminase and to analyse the role of glutamine transaminase in glutamine catabolism under microaerophilic conditions.

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