Role of Glutamine Aminotransferase in Glutamine Catabolism by
Saccharomyces cerevisiae under Microaerophilic Conditions

By MARIO SOBERÓN, JESÚS OLAMENDI, LAURA RODRÍGUEZ AND
ALICIA GONZÁLEZ*
Departamento de Biología Molecular de Plantas, Centro de Investigacion sobre Fijación de
Nitrógeno, UNAM, Apartado Postal 565-A, Cuernavaca, Morelos, Mexico

(Received 31 May 1989; accepted 6 June 1989)

The involvement of glutamine aminotransferase activity in glutamine catabolism by
Saccharomyces cerevisiae under microaerophilic conditions was studied. We were able to show
that there are at least two different glutamine aminotransferase activities that are differentiated
genetically, by their substrate specificity (pyruvate and glyoxylate dependence), and their
different modes of regulation. The pyruvate-dependent glutamine aminotransferase activity
plays a major role in glutamine catabolism under microaerophilic conditions since the wild-type
strain S288C showed a 10-fold higher activity in static cultures than in agitated ones. The same
strain also had 3-fold higher glutaminase B activity in agitated cultures than in static ones.
Pyruvate-dependent glutamine aminotransferase activity is not regulated directly by O₂ itself
since a p⁻ strain showed a high activity regardless of the extent of aeration of cultures. Finally,
we were able to isolate a mutant, strain CN20, derived from the p⁻ strain and unable to utilize
glutamine as the sole nitrogen source, which was severely affected in pyruvate-dependent but
not in glyoxylate-dependent aminotransferase activity.

INTRODUCTION

Glutamine plays a key role in nitrogen metabolism; it supplies nitrogen required for the
biosynthesis of a variety of nitrogenous metabolic intermediates in most organisms, including
Saccharomyces cerevisiae. Glutamine has also been proposed to be the co-repressor of nitrogen
catabolite repression in yeast (Dubois et al., 1977; Legrain et al., 1982). Due to the dual role that
glutamine plays, its biosynthesis and degradation may be a priori precisely regulated in order to
maintain an intracellular concentration that will support biosynthesis without resulting in
repression. Our previous work concerned the role of glutaminase (EC 3.5.1.2) in glutamine
assimilation in S. cerevisiae (Soberón & González, 1987a). We found that there are at least two
glutaminases (A and B) in this yeast and that these have different pH optima. Glutaminase A is
membrane bound whilst glutaminase B is cytoplasmic. Also, glutaminase B is negatively
modulated by organic acids such as pyruvate, glyoxylate and 2-oxoglutarate (Soberón &
González, 1987a). Additionally, the ω-amidase pathway constitutes an alternative route for
glutamine catabolism, and ω-amidase activity has been demonstrated in S. cerevisiae (Cooper &
Meister, 1977). In this pathway, glutamine is transaminated to yield different amino acids and
2-oxoglutarate, through the catalytic activity of glutamine aminotransferase; subsequently,
2-oxoglutarate is hydrolysed by ω-amidase to 2-oxoglutarate and ammonium. The existence
and function of the ω-amidase pathway in mammalian systems is well-documented (Cooper &
Meister, 1981). We have studied the ω-amidase pathway in S. cerevisiae, in which the highest
glutamine aminotransferase activity is found during growth in the presence of glutamate or
glutamine, with or without ammonium (Soberón & González, 1987b). This activity can be
detected using various 2-oxoacids (Soberón & González, 1987b). Our results suggest that the

Abbreviation: MM, Minimal medium.

0001-5612 © 1989 SGM
$\omega$-amidase pathway and the alternative pathway via glutaminase B are inversely regulated. That is, under microaerophilic conditions in which pyruvate accumulates, glutaminase B is inhibited and glutamine is degraded by the $\omega$-amidase pathway (Soberón & González, 1987b); under aerobic conditions, where the accumulation of pyruvate is less, glutamine is catabolized by glutaminase B. Our model predicts that a respiratory-deficient mutant strain should degrade glutamine through the $\omega$-amidase pathway regardless of the state of aeration of the culture.

In this paper we report the isolation of a mutant defective in glutamine aminotransferase which is unable to grow on glutamine as sole nitrogen source. The fact that this mutant was derived from a respiratory-deficient ($\rho^-$) strain indicates that the $\omega$-amidase pathway is the main catabolic pathway used to degrade glutamine under microaerophilic conditions in which anaerobic metabolism is favoured.

METHODS

Strains. The wild-type strain S288C (MATa; mal1; gal2) and strains DS400/A12 (MATa; met-; $\rho^-$) and AH22 (MATa; leu2-3; leu2-112; his3; can1) were obtained from the Cold Spring Harbor Laboratory, NY, USA.

Growth conditions. Strains were routinely grown in minimal media (MM) containing salts, trace elements and vitamins following the formula of Difco Yeast Nitrogen Base. Glucose (2%, w/v) was used as carbon source and 40 mM-$\left(\text{NH}_4\right)_2\text{SO}_4$ (i.e. 80 mM nitrogen), or 6.8 mM-glutamine, as nitrogen source. Cells were incubated at 30 °C with orbital shaking (175 r.p.m.). Static cultures were incubated at 30 °C without shaking. Growth was measured by determining the total protein content of cultures. Samples (20 ml) were filtered through 0.22 μm Millipore filters and cells were washed with distilled water. Resuspended samples were then precipitated with 2 ml trichloroacetic acid and centrifuged for 5 min at 2000 r.p.m. The pellets were suspended in 10 mM-NaOH and samples (0–0.2 ml) used for protein determination. This was done by the Lowry method using BSA as a standard.

Mutagenesis and mutant selection. To obtain mutants unable to use glutamine as nitrogen source, strain DS400/A12 was mutagenized by treatment with ethylmethane sulphonate (EMS), following the method of Fink (1970). After mutagenesis, the cells were allowed to grow for 24 h on MM plus 0.01% each of alanine, glycine, tyrosine and tryptophan with 40 mM-$\left(\text{NH}_4\right)_2\text{SO}_4$ as nitrogen source. These amino acids were added since their 2-oxoacids are substrates of glutamine aminotransferase, and an alteration in this activity could result in an auxotropic requirement for these compounds. Cells that could not use glutamine as a nitrogen source were then selectively enriched using the antibiotic N-glycosyl-polyfungin (Polaina & Conde, 1981). Cells were spread on the amino-acid-supplemented medium described above and incubated at 30 °C. Colonies which appeared after 3–5 d were replica-plated to MM plates containing 40 mM-$\left(\text{NH}_4\right)_2\text{SO}_4$ or 7.8 mM-glutamine as nitrogen source plus the above-mentioned amino acids. Cells which failed to grow on the glutamine plates were purified and further characterized.

Determination of glutaminase B activity. Soluble extracts were prepared by grinding whole cells in 50 mM-Tris/HCl buffer, pH 8.1, using a Braun homogenizer and glass beads. The extracts were centrifuged (20 min, 9000 g, 4 °C) and the supernatant was used as the source of enzyme.

The activity was assayed in reaction mixtures (0.5 ml) containing 50 mM-Tris/HCl, pH 8.1, 6 mM-$\left[U^{-14}\text{C}\right]$glutamate [0.045 μCi nmol$^{-1}$ (166.5 Bq nmol$^{-1}$)] and 0.1 ml of cell extract. The reaction mixture was incubated at 37 °C for 20 min or 40 min and then the reaction stopped by adding 1.5 ml cold 50 mM-Tris/HCl buffer. This mixture was passed through a Dowex 1 × 8-400 column and washed with 20 ml distilled water. $\left[U^{-14}\text{C}\right]$Glutamate was eluted from the column with 8 ml 2 M-acetic acid and a sample (0.1 ml) was used to determine $\left[U^{-14}\text{C}\right]$glutamate by liquid scintillation counting. Activity is expressed as nmol glutamate produced min$^{-1}$ (mg protein)$^{-1}$.

Determination of glutaminase aminotransferase activity. This was done following the method previously described (Soberón & González, 1987a). Activity is expressed as nmol 2-oxoglutaramate produced min$^{-1}$ (mg protein)$^{-1}$.

Determination of glutamate and glutamate pools. Cells were harvested by centrifugation (10 min, 7000 r.p.m.) and immediately suspended in 80% (v/v) ethanol and heated in boiling water for 10 min. Cells were then broken using a Braun cell disrupter and debris was removed by filtration through 0.22 μm Millipore filters. The filtrates containing the free amino acids were lyophilized and then resuspended in citrate (0.053 M) LiOH (0.3 M) buffer (pH 2.88). The amino acids were separated with an Amino Acid Analyzer and quantified in an Aminco ratio fluorimeter after coupling with o-phthalaldehyde.

Chemicals. All amino acids, EMS and bovine serum albumin were obtained from Sigma.

RESULTS AND DISCUSSION

Glutaminase B and glutamine aminotransferase activities in the $\rho^-$ strain

We have shown previously that glutaminase B activity is the major glutamine catabolic pathway when $S$. cerevisiae is cultured in aerobic conditions (Soberón & González, 1987a), but
Glutamine catabolism by yeast

Table 1. Glutaminase B and glutamine aminotransferase activities in extracts of strains S288C, DS400/A12 and CN20 grown statically or with agitation

Representative results of three experiments with a variation less than 15% are shown.

<table>
<thead>
<tr>
<th>Enzyme...</th>
<th>Glutaminase B</th>
<th>Glutamine aminotransferase</th>
<th>Glutaminase B</th>
<th>Glutamine aminotransferase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Agitated*</td>
<td></td>
<td>Static†</td>
<td></td>
</tr>
<tr>
<td>Strain</td>
<td>Pyruvate</td>
<td>Glyoxylate-</td>
<td>Pyruvate</td>
<td>Glyoxylate-</td>
</tr>
<tr>
<td></td>
<td>dependent</td>
<td>dependent</td>
<td>dependent</td>
<td>dependent</td>
</tr>
<tr>
<td>S288C (ρ+)</td>
<td>22.30</td>
<td>1-07</td>
<td>4-86</td>
<td>6-70</td>
</tr>
<tr>
<td>DS400/A12 (ρ−)</td>
<td>0-291</td>
<td>9-28</td>
<td>3-55</td>
<td>ND</td>
</tr>
<tr>
<td>CN20 (ρ−)</td>
<td>3-35</td>
<td>0-18</td>
<td>6-00</td>
<td>1-30</td>
</tr>
</tbody>
</table>

ND, Not detected.
* 240 r.p.m.: volume of medium/flask capacity = 1/6.
† Volume of medium/flask capacity = 1/2.

not under microaerophilic culture conditions (Soberón & González, 1987b). We therefore measured glutaminase B and glutamine aminotransferase activities in a wild-type strain (S288C) and in a ρ− strain (DS400/A12) cultured with glutamine as the sole nitrogen source under different conditions of aeration (Table 1).

Strain S288C had at least 3-fold lower glutaminase B activity in nonagitated cultures (Table 1) as was shown previously (Soberón & González, 1987a). Further, pyruvate-dependent glutamine aminotransferase activity was 10-fold higher in nonagitated cultures as compared to well-aerated cultures, whilst glyoxylate-dependent glutamine aminotransferase activity varied little with aeration conditions. These results indicate that pyruvate-dependent glutamine aminotransferase activity could play a major role in glutamine catabolism under microaerophilic culture conditions.

Strain DS400/A12 showed very low levels of glutaminase B activity regardless of aeration conditions (Table 1). This was expected since we previously proposed that glutaminase B activity is negatively regulated by some oxoacids, including pyruvate which accumulates under microaerophilic conditions (Soberón & González, 1987a); thus a ρ− strain would accumulate pyruvate regardless of the aeration conditions. In strain DS400/A12 pyruvate-dependent glutamine aminotransferase activity was similar in both well-aerated and nonagitated cultures. These results suggest that in the ρ− strain glutamine is catabolized by the pyruvate-dependent glutamine aminotransferase under aerobic and microaerophilic conditions, and that this activity is regulated by the metabolic status of the cell rather than directly by the O2 tension.

Isolation and characterization of a mutant impaired in pyruvate-dependent glutamine aminotransferase activity

Pyruvate-dependent glutamine aminotransferase activity appeared to play a major role in glutamine catabolism in the DS400/A12 (ρ−) strain regardless of culture conditions (see above). We therefore mutagenized this ρ− strain to look for mutants unable to grow on glutamine as sole nitrogen source. We isolated a mutant (CN20) which has significantly more-impaired growth on glutamine than on ammonium as sole nitrogen source (Fig. 1). Strain CN20 was mated with strain AH22, and diploids obtained were able to grow on glutamine showing that the mutation present was recessive (data not shown). Moreover, when the diploids were sporulated, the spores were non-viable. This phenotype could be due to a high intracellular glutamine content, since a mutant altered in glutaminase B shows a similar phenotype with respect to sporulation (Soberón & González, 1987a).

We measured glutaminase B and glutamine aminotransferase (pyruvate- and glyoxylate-dependent) activities in strain CN20 cultured on glutamine as sole nitrogen source. Table 1 shows that strain CN20 had a higher glutaminase B activity than DS400/A12 in both static and agitated cultures; nevertheless, these activities were significantly lower than those in the ρ+
Strain CN20 showed 5-fold lower and 25-fold lower pyruvate-dependent glutamine aminotransferase activity in agitated and static cultures respectively than its parent strain, but similar glyoxylate-dependent glutamine aminotransferase activities.

Since an impairment in glutamine catabolism should result in glutamine accumulation, we measured intracellular glutamine and glutamate pools in the strains CN20 and DS400/A12 grown on glutamine. CN20 had 3-fold and 4-fold higher glutamine and glutamate pools than DS400/A12. [0.200 and 0.068 pmol glutamine (mg protein)$^{-1}$ and 0.300 and 0.068 pmol glutamate (mg protein)$^{-1}$ for strains CN20 and DS400/A12 respectively. Cells grown for 12 h in agitated cultures with 0.1% (w/v) glutamine as sole nitrogen source.] This implies that growth impairment of CN20 is not due to a glutamate requirement. Glutamate accumulation in CN20 could be explained by the presence of glutaminase A, which we have shown previously to be responsible for glutamate accumulation in a mutant strain that lacks glutaminase B (Soberón & González, 1987a).

The results obtained suggest the existence of two different glutamine aminotransferase enzymes since the pyruvate- and the glyoxylate-dependent activities were regulated in different ways and the CN20 mutant strain was affected only in the pyruvate-dependent activity. Also, we have been able to show that the glyoxylate-dependent activity has at least two pH optima (data not shown). Further research is in progress to characterize the different glutamine aminotransferase activities with respect to substrate specificity and their role in glutamine catabolism. Nevertheless, the results presented suggest that the pyruvate-dependent glutamine aminotransferase activity is the major glutamine catabolic pathway in S. cerevisiae cultured under microaerophilic conditions.

The authors are grateful to Gloria Soberón for her critical review of the manuscript, to Concepción Hernández for typing the manuscript and to Herlinda Manrique for her secretarial assistance.

The work was supported in part by a grant from Fondo de Estudios de Investigaciones Ricardo J. Zevada.
REFERENCES


