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Regulation of Glutamine Synthesis by Glycine and Serine in *Neurospora crassa*

GEORGINA HERNÁNDEZ, YOLANDA MORA, AND JAIME MORA*

Centro de Investigación sobre Fijación de Nitrógeno. Universidad Nacional Autónoma de México. Cuernavaca, Morelos, Mexico

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The biosynthetic activities of the polypeptide subunits α and β of glutamine synthetase (GS) were inhibited in vitro by glycine and serine. These amino acids inhibited the growth of a mutant strain with partial GS activity when grown on glutamate as the nitrogen source and also blocked the synthesis of the glutamine in vivo, thus demonstrating the inhibitory effect on GS activity in vivo. Glycine and serine lowered the intracellular glutamine pool and regulated GSβ synthesis. A preferential induction of synthesis of the GSβ polypeptide was observed when either of these amino acids was present in the medium. On this basis, we obtained a glycine-sensitive mutant which showed a structural alteration of the GSβ polypeptide. The double regulatory effect of either glycine or serine on glutamine synthesis may be considered an example of the regulation of glutamine synthesis by α-amino nitrogen. It may be a mechanism that regulates the assimilation of ammonium into glutamate versus glutamine.

In *Neurospora crassa* more than one enzyme participates in the synthesis of glutamate and glutamine. Glutamate can be synthesized by either glutamate dehydrogenase (9) or glutamate synthase (10, 11). Glutamine synthetase (GS) occurs in two oligomeric forms composed of the α or the β monomers (4). The α and β monomers differ in their electrophoretic mobility (24). It has been suggested that these enzymes participate in different ammonium assimilation pathways (13).

Glutamine, an end product of nitrogen assimilation, has a key regulatory role in nitrogen metabolism in microorganisms. It has been shown that glutamine is a negative regulator of nitrogen catabolism in fungi (15, 29). In *N. crassa*, glutamine, in addition to being a substrate for transamidation reactions, is converted into α-amino groups of amino acids, α-ketoglutarate, and ammonium by the enzymes of the ω-amidase pathway. Glutamine transaminase synthesizes different amino acids and α-ketoglutaramate, and ω-amidase hydrolyzes this ketoacid to α-ketoglutarate and ammonium (2). Glutamine is also assimilated directly to α-amino nitrogen by the action of glutamate synthase which synthesizes two molecules of glutamate (1a). The ammonium released by the ω-amidase pathway is assimilated not only by glutamate dehydrogenase but also by GS, thus leading to the operation of a glutamine cycle in which this amino acid is continually degraded and resynthesized (2) (1a).

The regulation of GS, the enzyme that synthesizes glutamine, has been studied to a great extent. GS is regulated by the end products of glutamine metabolism (27). However, it is puzzling that the amino acids glycine and serine inhibit every GS tested so far, whether the GS comes from bacteria (7, 27), blue-green algae (cyanobacteria [23]), plants (18, 26), or animals (28). It is not obvious why these amino acids regulate GS activity, as it has not been well defined whether these amino acids really are products of glutamine catabolism.

To understand the physiological role of the inhibition by glycine and serine of GS from *N. crassa*, we studied the effect of these amino acids on the activity and synthesis of GS in vivo. Given that this enzyme occurs in two forms composed by two different monomers, we selected a glycine-sensitive mutant altered in the structure of one of the GS monomers, GSβ. We propose that the effect of these amino acids may be considered an example of the regulation of glutamine synthesis by α-amino nitrogen.

MATERIALS AND METHODS

Strains. The *N. crassa* wild-type strain 74A and the GS partial auxotroph *gln-1a* ($GS^{\pm}$) were obtained from the Fungal Genetics Stock Center at the Humbolt State University Foundation, Arcata, Calif. The GS partial auxotroph *gln-1b* (5) and the mutant strain *gln-1bR8*, which lacks the GSβ polypeptide ($GS\beta^{-}$) (3), were from the collection of J. Mora.

Growth conditions. Batch cultures of *N. crassa* were grown at 37°C on Vogel minimal medium (N) (31) containing 1.5% sucrose. Other nitrogen sources used in place of or in addition to ammonium nitrate are stated in the text. Growth was measured, as described previously (13, 30), by determining the total protein concentration by the method of Lowry et al. (14).

Mutagenesis and mutant selection. A conidial suspension of wild-type strain was adjusted to 2×10^7 conidia per ml, and 20 ml of this suspension was incubated with 0.2 mg of the mutagen *N*-methyl-*N'*-nitro-*N*-nitroso guanidine (Sigma Chemical Co., St. Louis, Mo.) for 45 min at room temperature with occasional agitation. The conidia were then centrifuged and washed twice with 20 ml of cold sterile water. The conidia treated in this way were enriched for glycine sensitivity by incubation (25°C for 72 h) in a 250-ml Erlenmeyer flask in 200 ml of minimal medium containing 25 mM NH_4NO_3 and 25 mM glycine as nitrogen sources. Every 12 h, the culture was filtered aseptically through cheese cloth into an Erlenmeyer flask containing fresh medium for 3 days. The enriched population was concentrated by filtration and

* Corresponding author.

then was washed, suspended in distilled water, and plated on N agar medium supplemented with 2% sorbose–0.05% fructose–0.05% dextrose–ammonium nitrate–glycine. The resistant conidia that started to grow were not considered, and then the Petri dishes were overlaid with 3 ml of 1.5% N agar supplemented with 10 mM glutamine as the nitrogen source. The new colonies which began to grow only after incubation on glutamine (glycine sensitive) at 29°C for 1 to 5 days in the dark were transferred to slants of N agar medium supplemented with 1.5% sucrose and 10 mM glutamine. Crosses of the glycine-sensitive colonies and the wild-type or the *gln-1b* mutant strain were done in 2× corn meal agar (Difco Laboratories, Detroit, Mich.). Spot testing and progeny analysis were carried out as described previously (6).

Determination of GS activity. Cell extracts were prepared as described previously (13, 30). GS was measured by its transferase and synthetase activities as described by Ferguson and Sims (8). Specific activity was expressed as units per milligram of protein; units represent micromoles of γ-glutamyl hydroxamate produced per minute. The biosynthetic GS activity was measured as reported by Prusiner and Milner (21) with the following modifications. The incubation mixture contained 50 mM imidazole, 0.5 mM EDTA, 4 mM L-glutamic acid, 8 mM NH_4Cl, 15 mM ATP, 80 mM $MgSO_4$, and 0.3 μCi of [^{3}H]glutamic acid, in a final volume of 0.1 ml and adjusted to pH 7.2. The reaction was started in parallel experiments by the addition of the enzyme. After incubation at 30°C, the reaction was stopped at various time intervals by the addition of 0.5 ml of 80% ethanol. Each mixture was then layered over Dowex 1X8 (200-400 mesh; 0.5 by 7-cm column) previously equilibrated with water. After elution with 3 ml of 30 mM glutamine, 1-ml fractions were collected, and the amount of radioactive glutamine in each fraction was determined in a liquid scintillation counter.

Purification of GS. Octameric GS, which is composed mainly of the β monomer, was purified as reported previously (19) from the wild-type strain 74A which had been grown on 5 mM glutamate (25°C for 8 h). Tetrameric GS, composed of the α monomer, was purified from a GSβ$^-$ mutant strain as reported previously (4). Cultures were grown on 5 mM glutamate for 36 h. The final preparation was dialyzed against extraction buffer B (5 mM KH_2PO_4, 0.5 mM EDTA, 50 mM K_2SO_4 [pH 7.2]).

Sucrose gradient sedimentation. Cell extracts were prepared either in buffer B or in buffer A (50 mM imidazole, 50 mM L-glutamic acid, 8 mM $MgSO_4$, 0.5 mM EDTA, 50 mM K_2SO_4, 5 mM 2-mercaptoethanol, 25 mM sodium bisulfite [pH 7.2]), as reported previously (13, 30). The samples were layered over a 5 to 20% continuous sucrose gradient and centrifuged (4°C, 12 h, 248,000 × *g*; SW-40 rotor) in a Beckman ultracentrifuge. After centrifugation, 25 fractions (0.3 ml each) were taken from the top of the tube, and GS transferase activity in each fraction was determined.

Immunoprecipitation of GS labeled in vivo. The cultures were labeled for 60 min with [^{35}S]methionine; 1 μCi/ml was used in cultures containing glutamine and 2 μCi/ml was used in those containing glutamine plus other amino acids as the nitrogen source.

The cell extracts were immunoprecipitated with polyclonal antibodies against purified GS prepared from the wild-type strain that contained both α and β monomers, as described previously (13).

Electrophoresis and fluorography. The immunoprecipitates were subjected to electrophoresis in a polyacrylamide slab gel containing sodium dodecyl sulfate and 7 M urea, as reported previously (24). The gel was stained with Coomassie blue and processed for fluorography (1).

Two-dimensional electrophoresis. Two-dimensional electrophoresis of the GS immunoprecipitates was done as described by O'Farrell (17), except that gels were run at 400 V for 18 h and the final high-voltage pulse was omitted; the second-dimension electrophoresis was carried out as reported previously (3).

Determination of amino acid pools. Samples for amino acid analysis were prepared by homogenizing conidia with 80% ethanol (10). The amino acids were separated by using an Aminco amino acid analyzer and, after being coupled with orthophthalaldehyde (Sigma), were quantified in an Aminco ratio fluorometer.

Glutamine labeling with [U-^{14}C]sucrose. Cultures were labeled for 30 min with [U-^{14}C]sucrose at 12 μCi/ml on 1 ml of media with the indicated nitrogen sources and 0.06% sucrose as the carbon source. The mycelia were collected, and the amino acid content was determined as described previously (10). The radioactivity incorporated into glutamine was determined in a liquid scintillation counter.

RESULTS

Effect of glycine and serine on glutamine synthesis in vitro and in vivo. It has been reported that glycine and serine inhibit the GS activity in *N. crassa* in vitro (12). We tested the inhibitory effect in vitro of these amino acids on each of the GS isozymes present in this fungi (4, 24). The activities of both GS isozymes which are composed of either α or β monomers were inhibited partially (up to 50%) by either glycine or serine (Fig. 1). The amino acid concentrations used to inhibit GS activity were similar to those used to inhibit GS from other microorganisms (27). There was a very slight difference in the degree of inhibition of the two GS isozymes; the activity of only the GS composed of α monomers was stimulated by low concentrations of glycine (Fig. 1). Stimulation of activity by low concentrations of inhibitors which are substrate analogs is well known (16). In the experiment shown in Fig. 1, low glutamate concentrations (4 mM) were used in the reaction mixture, since under saturating concentrations (20 mM) of glutamate the inhibition was not apparent (data not shown).

An inhibitory effect of glycine and serine on glutamine synthesis in vivo was evident only when a partial glutamine auxotroph (GS$^\pm$) was used. This auxotroph grew on glutamate at 37°C (5) but did not grow on glutamate and either glycine or serine (Fig. 2). In contrast these cells were able to grow after a lag phase on glutamine, the product of GS, plus an inhibitory amino acid (Fig. 2). The inhibition of glutamine synthesis in vivo by these amino acids was demonstrated by the barely detectable glutamine synthesis found when the GS$^\pm$ strain was grown on glutamate plus glycine (Table 1). Glutamine is continuously degraded and resynthesized when used as a nitrogen source (2), and a strain with a deficiency in GS synthesizes less glutamine than the wild-type strain (1a). Table 1 shows that the resynthesis of glutamine in vivo in this partial glutamine auxotroph on glutamine at 2 h of growth was lower than that on glutamate; this was expected since the synthesis of GS is repressed on glutamine (13, 22, 25). On glutamine-glycine the synthesis of glutamine in vivo was even lower (Table 1), and this may explain the lag phase that was observed (Fig. 2). Lower glutamine pools were present when the GS$^\pm$ mutant strain was grown on glutamine plus either glycine or serine than when grown on glutamine alone (Table 1). These may be explained by a lowered synthesis of glutamine when this mutant strain was grown on

glutamine-glycine and also by a reduction in glutamine uptake caused by the competition with different neutral amino acids (20). Under experimental conditions used in this study, lower glutamine uptake was obtained when either glycine or serine was added to the medium than when glutamine alone was used (data not shown).

Glycine and serine regulate GS synthesis. The inhibition of both glutamine uptake and GS activity by these inhibitory amino acids might lower the glutamine content which, in turn, would trigger GS synthesis (13, 22, 25). Therefore, the effect of these amino acids on the synthesis of α and β polypeptides of GS was studied.

When the wild-type strain was grown on glutamine in the presence of glycine or serine, there was only a minor effect on growth; higher GS specific activity and lower glutamine

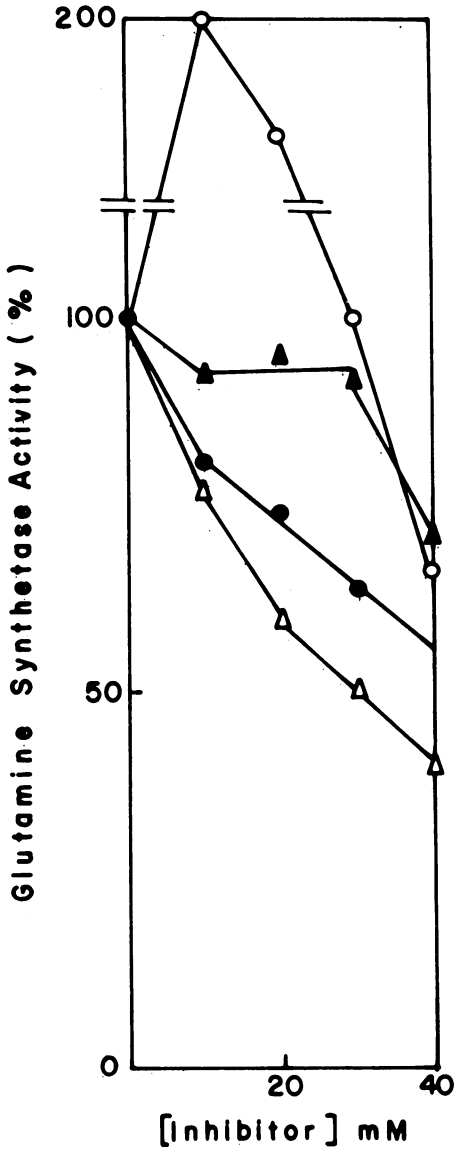

FIG. 1. Inhibition of GS activity in vitro. The activity of tetrameric GS composed of α polypeptides (open symbols) and octameric GS composed mainly of β polypeptide (dark symbols) were measured by the biosynthetic assay as described in the text. The inhibitory amino acids used were glycine (○, ●) and serine (△, ▲).

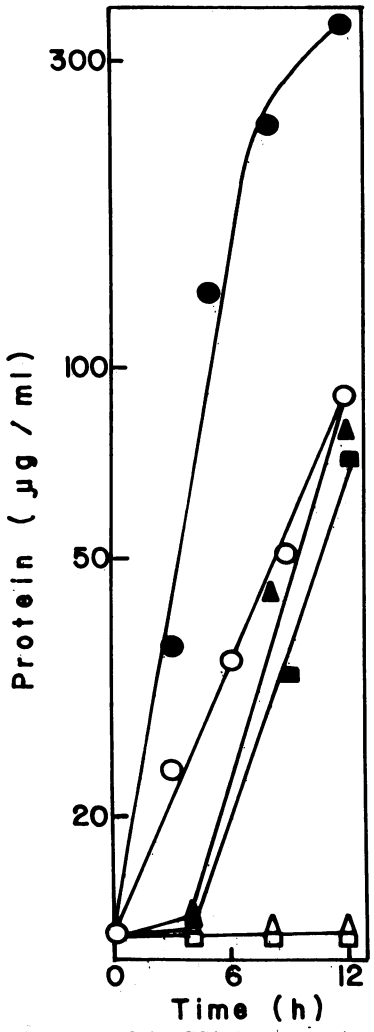

FIG. 2. Growth curves of the GS$^{\pm}$ mutant strain. The nitrogen sources used were 5 mM glutamate (○), glutamate–50 mM glycine (△), glutamate–50 mM serine (□), 5 mM glutamine (●), glutamine–50 mM glycine (▲), and glutamine–50 mM serine (■).

pools (Fig. 3A) were observed than when the wild-type strain was grown on glutamine alone. Leucine was used as a control because it competes with glutamine uptake in a manner similar to that of the inhibitory amino acids (20), but it is not an inhibitor of GS. In fact, leucine lowered the glutamine content and slightly increased GS activity when compared with glutamine alone (Fig. 3A). In the presence of any one of these three amino acids, GS synthesis increased. However, the inhibitory amino acids had an additional effect in that they preferentially increased the synthesis of GSβ over GSα monomers. This was not observed when either glutamine or glutamine-leucine were used. Under the latter condition, a higher synthesis of GSα over GSβ was observed (Fig. 3A). The higher proportion of GSβ monomer is indicated by the presence of the octameric GS (4). In cells grown on glutamine-glycine, an oligomer which corresponded to an octamer was observed; this GS was different from the broad oligomeric form found in cells grown on glutamine (Fig. 3B).

Selection of a glycine-sensitive mutant altered in GS. The inhibitory effect of glycine and serine on GS activity, as well as their preferential effect on the rate of synthesis of the GSβ monomer, enabled us to predict that a mutant strain that was

TABLE 1. Synthesis and pools of glutamine in vivo in the GS$^{\pm}$ strain

Nitrogen source[a]	Glutamine pool (μmol/mg of protein)[b]	Glutamine specific radioactivity (cpm/μmol)[c]
Glutamate	0.053	91,678[d]
Glutamate-glycine	0.026	838[d]
Glutamate-serine	0.029	
Glutamine	0.195	6,640[e]
Glutamine-glycine	0.056	2,278[e]
Glutamine-serine	0.074	

[a] The amino acid concentrations used were 5 mM glutamate, 5 mM glutamine, 50 mM glycine, and 50 mM serine.
[b] Determined at 12 h of incubation.
[c] To determine the synthesis of glutamine in vivo, the cultures received a pulse with [U-^{14}C]sucrose, as described in the text.
[d] Determined at 10 h of incubation.
[e] Determined at 2 h of incubation.

glycine sensitive in the presence of ammonium might have a structural mutation which would alter the β monomer activity. One of the glycine-sensitive mutants selected, strain *gln-1k*, grew suboptimally on ammonium or glutamate as nitrogen sources and did not grow at all when glycine was added. It grew optimally on glutamine and more slowly on glutamine-glycine (data not shown). Mutant *gln-1k* was crossed with mutant strain *gln-1b*, which has a different structural alteration in the GSβ polypeptide (3). The recombination frequency found was lower than 1.2×10^{-5}, implying that the two mutations are allelic.

The α and β monomers of GS differ in their electrophoretic mobility (24). Figure 4 shows the polypeptides and the oligomeric forms of GS from strain *gln-1k* compared with those of the wild-type GS. The isoelectric point of the GSβ polypeptide of the glycine-sensitive mutant was altered, whereas the isoelectric point of the GSα polypeptide remained the same as that of the GSα of the wild type (Fig. 4A). Strain *gln-1k* had low GS activity, and the enzyme was more sensitive in vitro to glycine inhibition (data not shown). In a sucrose gradient its oligomeric form migrated to a position between those of the octameric and the tetrameric forms (Fig. 4B). The structural alteration in GSβ found in this glycine-sensitive mutant was different from the alterations reported for several glutamine auxotrophs (3). Because of an alteration in the GSβ monomer, there was a lower GS activity, which may be the cause of the glycine sensitivity. These data support the interpretation that a highly active octameric GS, composed mainly of the β monomer, is required to relieve the inhibitory effect of glycine.

DISCUSSION

In *N. crassa* glycine and serine inhibited the synthesis of glutamine both in vitro and in vivo. The biosynthetic activity in vitro of both isozymes, composed of either α or β monomers, was partially inhibited by glycine and serine (Fig. 1). We found that the inhibitory effect depends on the concentration of glutamate. Total growth inhibition of a GS$^{\pm}$ mutant strain was observed when either glycine or serine was added to the medium containing glutamate, a substrate of GS (Fig. 2). Under such conditions, the synthesis of glutamine decreased 100-fold, even though the content of glutamine only decreased by half (Table 1). These data suggest that the synthesis of glutamine in vivo, rather than the absolute content of glutamine, plays an important role for optimal cell growth; studies that are in progress attempt to explain this phenomenon. The addition of glutamine, the product of GS, reversed the effect of glycine and serine. The GS$^{\pm}$ mutant strain was able to grow on glutamine and an inhibitory amino acid (Fig. 2).

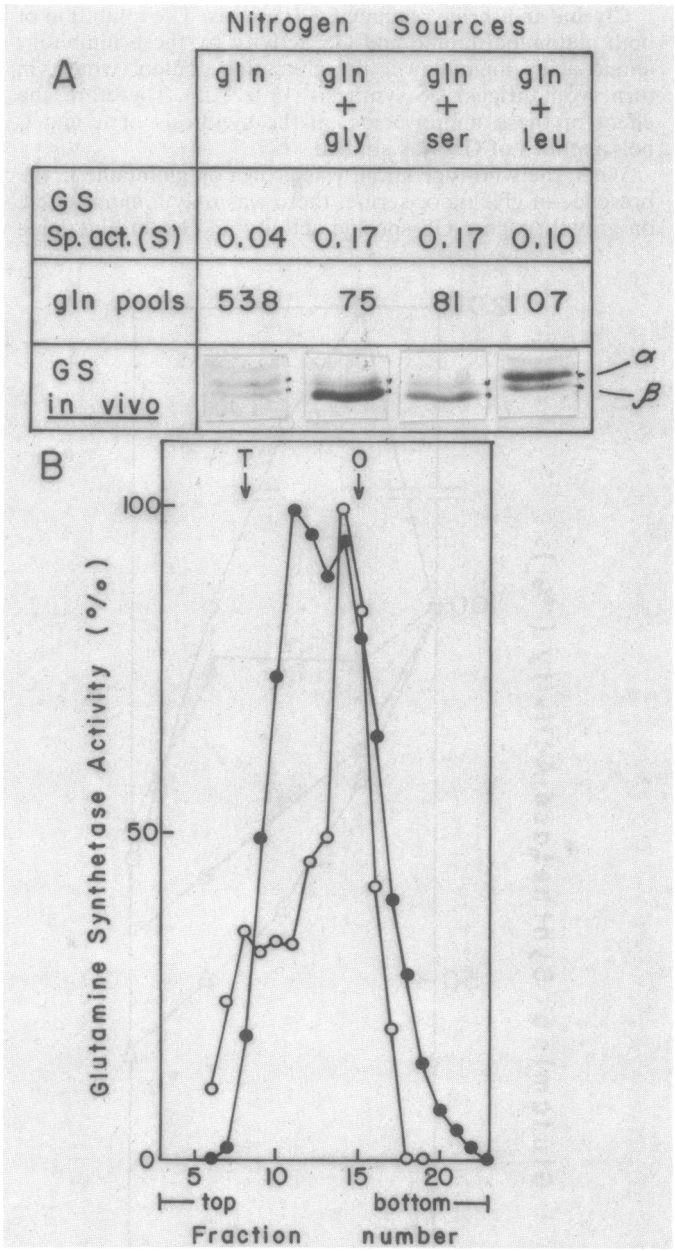

FIG. 3. Specific activity, synthesis in vivo, and oligomeric forms of GS and glutamine pools from the wild-type strain grown on different nitrogen sources. (A) Cultures were grown for 10 h on 5 mM glutamine (gln) or on glutamine and 50 mM glycine (gly), 50 mM serine (ser), or 50 mM leucine (leu) as nitrogen sources. Specific activity of GS synthetase (S) from dialyzed cell extracts is expressed as units per milligram of protein. Glutamine intracellular pools at 10 h are expressed as nanomoles per milligram of protein. Fluorography is of the GS region of acrylamide gels of GS immunoprecipitates labeled in vivo. (B) Sucrose gradient sedimentation of GS from cultures grown on glutamine (●) or on glutamine-glycine (○). Cell extracts were prepared in buffer B. GS transferase activity was normalized to that of the peak fraction. The positions of the tetramer (T) and the octamer (O) of GS are indicated.

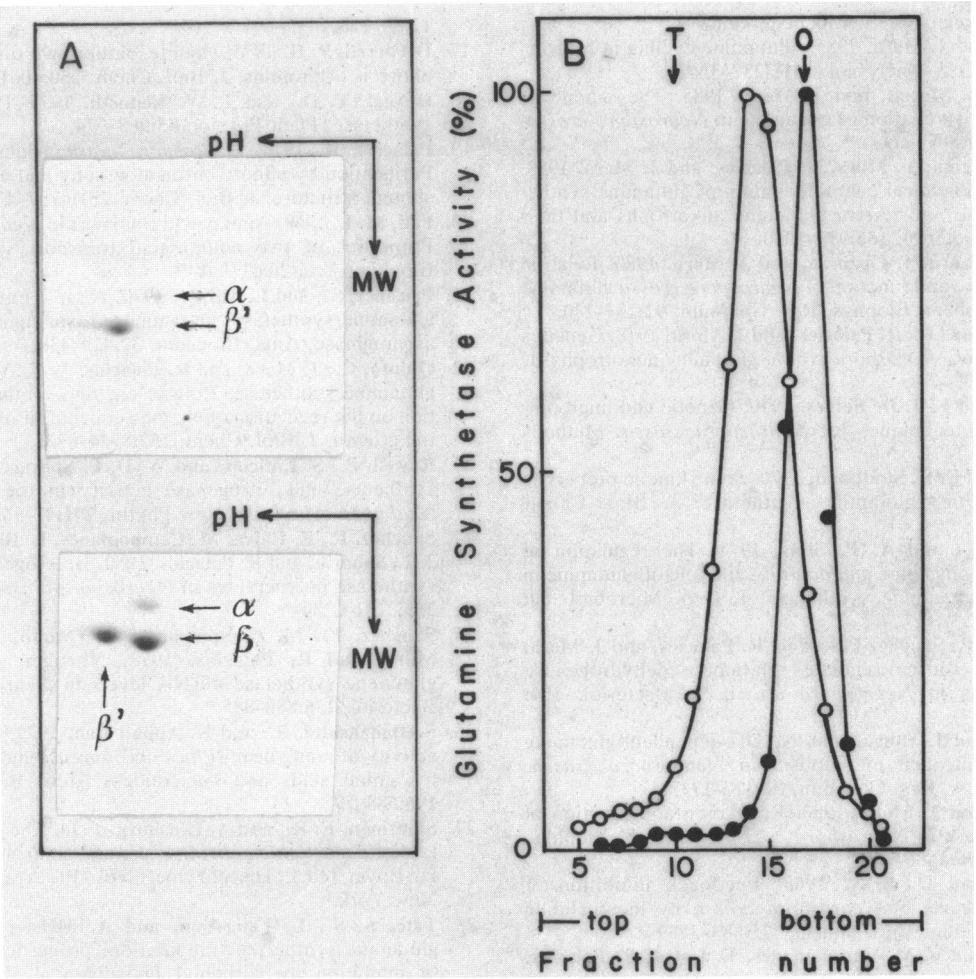

FIG. 4. Characterization of GS polypeptides and GS oligomeric forms from a glycine-sensitive mutant. Strains were grown for 24 h at 25°C on glutamate as nitrogen source. (A) Immunoprecipitates of GS from cell extracts were subjected to two-dimensional electrophoresis and stained with Coomassie blue. (Top panel) *gln-1k* mutant strain. (Bottom panel) *gln-1k* extract mixed with wild-type strain extract. The first (pH) and second (molecular weight [MW]) dimensions and positions of GS polypeptides are indicated. (B) Sucrose gradient sedimentation of GS from wild-type (●) and *gln-1k* (○) strains. Cell extracts were prepared in buffer A. GS transferase activity was normalized to that of the peak fraction. The positions of the tetramer (T) and the octamer (O) of GS are indicated.

When glycine or serine was present, the synthesis of the GSβ polypeptide, the main component of the highly active octameric GS, was preferentially induced (Fig. 3), thus leading to optimal growth in the presence of glycine or serine. Leucine, which competes with glutamine uptake but is not a GS inhibitor, induced synthesis of the GSα monomer (Fig. 3) which forms the less active tetrameric isozyme (3). One would expect that a mutant strain with an altered GSβ monomer would be sensitive to glycine. Our results confirm this prediction (Fig. 4).

All the GS mutants previously described have been the products of a single mutation in the GSβ polypeptide that gives rise to glutamine auxotrophy through the inactivation of both GS polypeptides; very few mutations in the β gene give this phenotype (3). We propose that the selection procedure used here, that is, to look for glycine-sensitive mutants, opens up the possibility of obtaining many different structural alterations in GSβ.

The experimental evidence presented here on the effects of glycine and serine, i.e., the inhibition of growth of the $GS^{\pm}$ mutant strain when grown on glutamate (Fig. 2), the reduction of glutamine synthesis in vivo when the $GS^{\pm}$ strain is grown on glutamate (Table 1), and the isolation of a glycine-sensitive mutant with an altered GSβ (Fig. 4), demonstrate the inhibition of glutamine synthesis in vivo by these amino acids which are partial inhibitors of GS (Fig. 1).

Ammonium can be assimilated either into glutamate by glutamate dehydrogenase or into glutamine by GS; nitrogen is distributed either by transamination or transamidation. We propose that the double regulatory effect of glycine and serine on glutamine synthesis, described above, is a mechanism by which an optimal relation between glutamate and glutamine may be achieved. Initially, GS inhibition would favor ammonium assimilation into glutamate and amino acids; however, by lowering the glutamine pool, GS synthesis would be induced to reestablish glutamine synthesis.

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LITERATURE CITED

1. **Bonner, W. M., and R. A. Laskey.** 1974. A film detection method for tritium-labeled proteins and nucleic acids in

polyacrilamide gels. Eur. J. Biochem. **46**:83–88.
1a. **Calderon, J., and J. Mora.** 1985. Glutamine cycling in Neurospora crassa. J. Gen. Microbiol. **131**:3237–3242.
2. **Calderón, J., E. Morett, and J. Mora.** 1985. The ω-amidase pathway in the degradation of glutamine in *Neurospora crassa*. J. Bacteriol. **161**:807–809.
3. **Dávila, G., S. Brom, Y. Mora, R. Palacios, and J. Mora.** 1983. Genetic and biochemical characterization of glutamine synthetase from *Neurospora crassa* glutamine auxotrophs and their revertants. J. Bacteriol. **156**:993–1000.
4. **Dávila, G., M. Lara, J. Guzmán, and J. Mora.** 1980. Relation between structure and function of *Neurospora crassa* glutamine synthetase. Biochem. Biophys. Res. Commun. **92**:134–140.
5. **Dávila, G., F. Sánchez, R. Palacios, and J. Mora.** 1978. Genetics and physiology of *Neurospora crassa* glutamine auxotrophs. J. Bacteriol. **134**:693–698.
6. **Davis, R. H., and F. J. De Serres.** 1970. Genetic and microbiological research techniques for *Neurospora crassa*. Methods Enzymol. **17**:79–143.
7. **Deuel, T. F., and E. R. Stadtman.** 1970. Some kinetic properties of *Bacillus subtilis* glutamine synthetase. J. Biol. Chem. **245**:5206–5213.
8. **Ferguson, A. R., and A. P. Sims.** 1974. The regulation of glutamine metabolism in *Candida utilis*: the role of glutamine in the control of glutamine synthetase. J. Gen. Microbiol. **80**: 159–171.
9. **Hernández, G., R. Sánchez-Pescador, R. Palacios, and J. Mora.** 1983. Nitrogen source regulates glutamate dehydrogenase NADP synthesis in *Neurospora crassa*. J. Bacteriol. **154**: 524–528.
10. **Hummelt, G., and J. Mora.** 1980. NADH-dependent glutamate synthase and nitrogen metabolism in *Neurospora crassa*. Biochem. Biophys. Res. Commun. **92**:127–133.
11. **Hummelt, G., and J. Mora.** 1980. Regulation and function of glutamate synthase in *Neurospora crassa*. Biochem. Biophys. Res. Commun. **96**:1688–1694.
12. **Kapoor, M., and D. Bray.** 1968. Feedback inhibition of glutamine synthetase of *Neurospora crassa* by nicotinamide adenine dinucleotides. Biochemistry **7**:3583–3589.
13. **Lara, M., L. Blanco, M. Campomanes, E. Calva, R. Palacios, and J. Mora.** 1982. Physiology of ammonium assimilation in *Neurospora crassa*. J. Bacteriol. **150**:105–112.
14. **Lowry, O. H., N. J. Rosenbrough, A. L. Farr, and R. J. Randall.** 1951. Protein measurement with the folin phenol reagent. J. Biol. Chem. **193**:256–275.
15. **Marzluf, G. A.** 1981. Regulation of nitrogen metabolism and gene expression in fungi. Microbiol. Rev. **45**:437–461.
16. **Monod, J., J. Wyman, and J. P. Changeux.** 1965. On the nature of allosteric transition: a plausible model. J. Mol. Biol. **12**:88–118.
17. **O'Farrell, P. H.** 1975. High resolution two-dimensional electrophoresis of proteins. J. Biol. Chem. **250**:4001–4021.
18. **O'Neal, T. D., and J. W. Kenneth.** 1975. Pea leaf glutamine synthetase. Plant Physiol. **55**:968–974.
19. **Palacios, R.** 1976. *Neurospora crassa* glutamine synthetase. Purification by affinity chromatography and characterization of subunit structure. J. Biol. Chem. **251**:4787–4791.
20. **Pall, M. L.** 1969. Amino acid transport in *Neurospora crassa*. I. Properties of two amino acid transport systems. Biochim. Biophys. Acta **203**:139–149.
21. **Prusiner, S., and L. Milner.** 1970. A rapid radioactive assay for glutamine synthetase, glutaminase, asparagine synthetase and asparaginase. Anal. Biochem. **37**:429–438.
22. **Quinto, C., J. Mora, and R. Palacios.** 1977. *Neurospora crassa* glutamine synthetase. Role of enzyme synthesis and degradation on the regulation of enzyme concentration during exponential growth. J. Biol. Chem. **252**:8724–8727.
23. **Rowell, P., S. Enticott, and W. D. P. Stewart.** 1977. Glutamine synthetase and nitrogenase activity in the blue green alga *Anabaena cylindrica*. New Phytol. **791**:41–54.
24. **Sánchez, F., E. Calva, M. Campomanes, L. Blanco, J. Guzmán, J. L. Saborío, and R. Palacios.** 1980. Heterogeneity of glutamine synthetase polypeptides in *Neurospora crassa*. J. Biol. Chem. **255**:2231–2234.
25. **Sánchez, F., M. Campomanes, C. Quinto, W. Hansberg, J. Mora, and R. Palacios.** 1978. Nitrogen source regulates glutamine synthetase mRNA levels in *Neurospora crassa*. J. Bacteriol. **136**:880–885.
26. **Seethalakshmi, S., and N. Appaji Rao.** 1979. Regulation of the activity of mung bean (*Phaseolus aureus*) glutamine synthetase by amino acids and nucleotides. Arch. Biochem. Biophys. **196**:588–597.
27. **Stadtman, E. R., and A. Ginsburg.** 1974. The glutamine synthetase of *Escherichia coli*: structure and control, p. 755–807. *In* P. D. Boyer (ed.), The enzymes, vol. 10. Acadmic Press, Inc., New York.
28. **Tate, S. S., L. Fang-Yun, and A. Meister.** 1972. Rat liver glutamine synthetase. Preparation, properties and mechanism of inhibition by carbamyl phosphate. J. Biol. Chem. **247**: 5312–5321.
29. **Vaca, G., and J. Mora.** 1977. Nitrogen regulation of arginase in *Neurospora crassa*. J. Bacteriol. **131**:719–725.
30. **Vichido, I., Y. Mora, C. Quinto, R. Palacios, and J. Mora.** 1978. Nitrogen regulation of glutamine synthetase in *Neurospora crassa*. J. Gen. Microbiol. **106**:251–259.
31. **Vogel, H. J.** 1956. A convenient growth medium for *Neurospora* (medium N). Microbiol. Genet. Bull. **13**:42–43.