Glutamine Assimilation Pathways in *Neurospora crassa* Growing on Glutamine as Sole Nitrogen and Carbon Source

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Neurospora crassa wild-type is almost unable to grow on glutamine as sole nitrogen and carbon source but a GDH⁻;GS[±] double mutant strain, lacking NADP-dependent glutamate dehydrogenase and partially lacking glutamine synthetase did grow. Under these conditions, the double mutant had a higher chemical energy content than the wild-type. Enzyme assays and labelling experiments with glutamine indicated that in the double mutant glutamine was degraded to ammonium and to carbon skeletons by glutamate synthase, the catabolic (NADH-dependent) glutamate dehydrogenase and the glutamine transaminase- ω -amidase pathway.

INTRODUCTION

Since glutamine is a nitrogen donor and the final product of ammonium assimilation in micro-organisms, it plays a central metabolic role as a repressor of nitrogen catabolism. It has been shown that in *Neurospora crassa* glutamine is a negative regulator of nitrogen catabolism (Vaca & Mora, 1977; Marzluf, 1981). Glutamine synthetase (GS) (EC 6.3.1.2) is composed of two polypeptides (Sánchez *et al.*, 1980; Dávila *et al.*, 1980) and glutamine regulates the level of specific enzyme synthesis (Vichido *et al.*, 1978), which correlates with the amount of mRNA (Sánchez *et al.*, 1978; Lara *et al.*, 1982). The carbon source also participates by regulating GS degradation (Mora *et al.*, 1980).

In *N. crassa*, glutamine, in addition to being assimilated by transamidation reactions, is converted into 2-oxoglutarate and ammonium by the enzymes of the glutamine transaminase- ω -amidase pathway (Calderón *et al.*, 1985). In this pathway, glutamine is transaminated to yield different amino acids and 2-oxoglutarate through the participation of a glutamine transaminase (T-Gln). Subsequently, the 2-oxoglutarate is hydrolysed to 2-oxoglutarate and ammonium by the action of an ω -amidase (Calderón *et al.*, 1985). In this fungus, glutamine is also assimilated directly to α -amino nitrogen by the action of glutamate synthase (GOGAT) (EC 1.4.1.14), which synthesizes two molecules of glutamate (Calderón & Mora, 1985). The ammonium released by the glutamine transaminase- ω -amidase pathway is assimilated by NADP-dependent glutamate dehydrogenase (NADP-GDH) (EC 1.4.1.4) and also by GS, thus leading to the operation of a glutamine cycle (Calderón & Mora, 1985). GOGAT also participates in cycling glutamine to glutamate (Calderón & Mora, 1985). As a result of the glutamine cycle, glutamate and glutamine are continually resynthesized (Calderón & Mora, 1985) at the expense of carbon skeletons, reductive power and ATP. No glutaminase activity has been detected in *N. crassa* (Calderón *et al.*, 1985). We have reported (Hernández & Mora, 1986) that cycling is necessary to maintain an optimal carbon flow from sucrose.

Abbreviations: AOA, aminooxyacetic acid; DON, 6-diazo-5-oxo-L-norleucine; GDH, glutamate dehydrogenase; GOGAT, glutamate synthase; GS, glutamine synthetase; T-Gln, glutamine transaminase.

It has been shown (Calderón & Mora, 1985) that for glutamine to be optimally assimilated as a nitrogen source, it must be converted to glutamate, the other nitrogen donor. The activity of GOGAT and the incorporation of the ammonium released by the glutamine transaminase- ω -amidase pathway through the NADP-GDH are essential to synthesize glutamate, since the contribution of amidotransferases other than GOGAT is a minor component of the net assimilation of glutamine into glutamate (Calderón & Mora, 1985).

Glutamine cycling operates in the presence of ammonium as nitrogen source as indicated by the presence of 2-oxoglutamate under such conditions (Calderón *et al.*, 1985). Studies using ^{13}N as a tracer have also shown the operation of glutamine cycling in *N. crassa*, where most of the nitrogen is retained cycling between glutamate and glutamine (Calderón *et al.*, 1989).

N. crassa is almost unable to grow on glutamine as sole nitrogen and carbon source (Mora *et al.*, 1980). We propose that this may be explained by the great expenditure of energy as a result of glutamine cycling (Calderón & Mora, 1985), and also as a result of the trapping of carbon skeletons in the continuous synthesis of glutamate and glutamine. In this paper we report that in conditions where glutamate and glutamine synthesis are restricted, *N. crassa* can spare carbon skeletons and chemical energy, which in turn allows this fungus to grow on glutamine as the sole nitrogen and carbon source. In addition, the pathways for glutamine assimilation for use as a nitrogen and a carbon source have been identified.

METHODS

Strains. All stocks came from the collection of J. Mora or from the Fungal Genetics Stock Center, Humbolt State University, Arcata, California, USA. The basic stocks were the wild-type strain 74-A; the *am-1* strain lacking glutamate dehydrogenase (GDH⁻) (Fincham, 1950); the *gln-1a* strain partially lacking glutamine synthetase activity (GS[±]) (Dávila *et al.*, 1978); the double mutant strain *am-1; gln-1a* (GDH⁻; GS[±]); and the *en-am-2* strain (GOGAT⁻) that lacks GOGAT activity (Hummelt & Mora, 1980b).

Growth conditions. Cultures of *N. crassa* were grown on Vogel's minimal medium (Vogel, 1964) supplemented with 5 mM-glutamine, instead of ammonium nitrate, as the nitrogen source, at 30 °C with shaking as indicated. The carbon source used is stated in the text.

Growth was measured as described previously (Vichido *et al.*, 1978) by determining the total protein concentration by the Lowry method.

Determination of amino acid pools. Samples for amino acid analysis were prepared by homogenizing conidia with 80% (v/v) ethanol (Espín *et al.*, 1979). The amino acids were separated in an Aminco Amino Acid Analyzer, coupled with *ortho*-phthalaldehyde (Sigma) and quantified in an Aminco ratio fluorimeter.

Ammonium determination. Ammonium concentration in samples of medium was measured with an Orion electrode (Espín *et al.*, 1979).

Determination of nucleotides. After 8 h growth, 300 ml of the culture medium were quickly filtered and immersed in liquid nitrogen, and the nucleotides were extracted by resuspending the samples in 0.6 M-HClO₄. The extracts were centrifuged at 10000 r.p.m. for 2 min, and the supernatants were adjusted to pH 7–7.5 by adding 1.0 M-KOH with rapid mixing. The insoluble KClO₄ was removed by centrifugation. The nucleotides were separated and quantified by high-performance liquid chromatography on a Radial Pak sax-cartridge (Waters). The chromatographic conditions were essentially the same as those used by McKeag & Brown (1978).

Determination of enzyme activities. Cell-free extracts of *N. crassa* were prepared as described previously (Dávila *et al.*, 1978). GS activity was measured by its synthetase activity as described by Ferguson & Sims (1974). Catabolic NAD-dependent glutamate dehydrogenase (NAD-GDH) and NADP-GDH were measured as described by Fincham (1950). GOGAT was measured as described by Hummelt & Mora (1980a). T-Gln was measured as described by Calderón *et al.* (1985).

Measurement of CO₂ from [U-¹⁴C]glutamine. Samples of cultures grown for 12 h on 5 mM-glutamine as sole nitrogen and carbon source were transferred to a side-arm tube with fresh medium without glutamine, and preincubated for 15 min with or without the inhibitors aminooxyacetic acid (AOA; 10 mM) and 6-diazo-5-oxo-L-norleucine (DON; 1 mM). These cultures were then given a 1 h pulse with [U-¹⁴C]glutamine [0.2 µCi ml⁻¹ (7.5 kBq ml⁻¹)] and unlabelled glutamine to give a final concentration of 0.5 mM. The CO₂ released was absorbed with NaOH (Mora *et al.*, 1972). When the CO₂ released was measured continually during 12 h growth, the cultures were grown on 5 mM-glutamine with [U-¹⁴C]glutamine [0.2 µCi ml⁻¹ (7.5 kBq ml⁻¹)] with shaking at 250 r.p.m. (OD₅₄₀ of inoculum 0.1).

Glutamine labelling with [U-¹⁴C]glutamate. Conidia from the wild-type, GS[±] and GDH⁻;GS[±] strains were germinated for 12 h in a medium supplemented with 5 mM-glutamine. The mycelium was collected by filtration

and transferred to a medium containing 5 mM-glutamine and 0.5 $\mu\text{Ci ml}^{-1}$ (18.75 kBq ml^{-1}) [$U\text{-}^{14}\text{C}$]glutamate. After 90 min incubation the mycelium was collected and the amino acid content was determined as reported by Espin *et al.* (1979); the radioactivity incorporated in each amino acid was determined in a scintillation counter.

Reproducibility of results. The experiments reported were each repeated at least once; representative results are shown.

RESULTS

Growth of the wild-type strain and the double mutant strain GDH⁻;GS[±] on glutamine as a nitrogen and carbon source

The *N. crassa* wild-type strain (74-A) grew very slowly on glutamine as a nitrogen and carbon source (Fig. 1*a*). Previously, we described a glutamine cycle in which glutamine is continually degraded and resynthesized in *N. crassa* (Calderón & Mora, 1985; Calderón *et al.*, 1989). This cycle consumes NADPH and ATP for glutamate and glutamine synthesis, respectively. On this basis, we tested the capacity of the double mutant strain, lacking NADP-GDH activity and partially lacking GS activity (GDH⁻;GS[±]), to grow on glutamine as the sole nitrogen and carbon source. The double mutant strain did grow but the growth rate was slower than that on glutamine plus sucrose (Fig. 1*a*). The growth rate of the double mutant strain increased when the culture was optimally aerated by lowering the inoculum size (Fig. 1*b*). Even the wild-type strain could grow on glutamine as nitrogen and carbon source if the culture was inoculated at low cell density (Fig. 1*b*). The aeration was also modified by shaking at a low speed. When the wild-type and the double mutant strains were incubated with shaking at 100 r.p.m., their growth rates were lower even with a low inoculum (Fig. 1*c*). Under these conditions the double mutant strain showed a similar growth rate on glutamine as nitrogen and carbon source as on glutamine plus sucrose; however, the wild-type grew only on glutamine plus sucrose (Fig. 1*c*).

Chemical energy content, glutamine cycling enzymes, amino acid content and ammonium excretion

The NADH, NADPH, ATP and ADP contents were higher in the GDH⁻;GS[±] double mutant strain compared to the wild-type strain, when these strains were incubated on glutamine as nitrogen and carbon source (Fig. 2). The difference in total chemical energy was almost 200% when comparing the double mutant strain with the wild-type strain (Fig. 2). The activities of the enzymes involved in glutamine cycling were measured. NAD-GDH activity was also determined since the conversion of glutamine to glutamate would supply this amino acid which can then be catabolized by NAD-GDH for the production of 2-oxoglutarate. As reported previously (Dantzig *et al.*, 1978), NAD-GDH and NADP-GDH are regulated by the carbon source. Table 1 shows that on glutamine as nitrogen and carbon source the wild-type strain had a low biosynthetic NADP-GDH activity, although this was at least 14-fold higher than in the double mutant strain. However, under the same conditions the catabolic NAD-GDH activity of the double mutant was 62% greater than that in the wild-type. The GDH⁻;GS[±] double mutant strain catabolized glutamine efficiently, as indicated by the lower glutamine content (Table 2).

GOGAT also participates in glutamine assimilation. As shown in Table 1, GOGAT activity was 64% higher in the GDH⁻;GS[±] mutant strain on glutamine without sucrose compared to the wild-type strain. In such conditions GOGAT activity is expressed despite the presence of a glutamate pool (Table 2) which has been shown to repress GOGAT in *N. crassa* (Hummelt & Mora, 1980*b*; Lomnitz *et al.*, 1987). As already reported (Dávila *et al.*, 1978), the single mutant GS[±] strain has 10-fold less GS activity than the wild-type strain. This was also true for the GDH⁻;GS[±] double mutant strain growing in the presence of sucrose (Table 1). Similar to NADP-GDH, GS activity was lower in the wild-type strain on glutamine compared to glutamine plus sucrose. Nevertheless, GS activity in the wild-type strain was 250% of that in the GDH⁻;GS[±] mutant strain growing on glutamine as nitrogen and carbon source.

In the GDH⁻;GS[±] strain growing on glutamine alone as carbon and nitrogen source, 2-oxoglutarate could be supplied to the tricarboxylic acid cycle through the operation of GOGAT and the catabolic NAD-GDH activities (Table 1).

Fig. 1. Growth curves of the wild-type (○, ●), $GDH^-;GS^\pm$ (□, ■), $GS^\pm$ (▲) and GDH^- (▼) strains from cultures grown on 5 mM-glutamine with (open symbols), or without (filled symbols) 1.5% (w/v) sucrose. The cultures were grown as follows: (a) shaking at 250 r.p.m., inoculum $OD_{540} = 0.1$; (b) shaking at 250 r.p.m., inoculum $OD_{540} = 0.05$; (c) shaking at 100 r.p.m., inoculum $OD_{540} = 0.05$.

Table 1. *Specific enzyme activities of the wild-type and $GDH^-;GS^\pm$ strains*

The specific enzyme activities were determined after 12 h growth on 5 mM-glutamine with or without 1.5% (w/v) sucrose as carbon source. The cultures were grown with shaking at 250 r.p.m.; the inoculum had an OD_{540} of 0.1. ND, Not detected.

Strain	Sucrose	Specific activity [nmol min ⁻¹ (mg protein) ⁻¹]				
		GOGAT	T-Gln	NAD-GDH	NADP-GDH	GS
Wild-type	+	10.9	3.9	18.3	804.0	6.0
	-	8.1	3.4	2130.0	14.0	1.0
$GDH^-;GS^\pm$	+	10.6	2.8	18.9	ND	0.6
	-	13.3	5.5	3440.0	ND	0.4

Fig. 2. Chemical energy content of the wild-type strain (□) and the GDH⁻;GS[±] double mutant strain (■) growing on glutamine as sole nitrogen and carbon source. The nucleotides were determined after 8 h incubation. Chemical energy is expressed as the sum of ATP equivalents (1, 1/2, 3 and 4 for ATP, ADP, NADH and NADPH respectively). The cultures were inoculated at an OD₅₄₀ of 0.1 and grown with shaking at 250 r.p.m.

Table 2. *Glutamine and glutamate content of the wild-type, GS[±], and GDH⁻;GS[±] strains growing on 5 mM-glutamine as sole nitrogen and carbon source*

Amino acid contents were determined after 12 h growth. The cultures were grown with shaking at 250 r.p.m.; the inoculum had an OD₅₄₀ of 0.1.

Strain	Amino acid	Amino acid content [μmol (mg protein) ⁻¹]
Wild-type	Glutamate	0.3
	Glutamine	1.5
GS [±]	Glutamate	0.5
	Glutamine	1.1
GDH ⁻ ;GS [±]	Glutamate	0.3
	Glutamine	0.4

Table 3. *Ammonium excretion by the wild-type, GDH⁻, GS[±] and GDH⁻;GS[±] strains growing on 5 mM-glutamine as sole nitrogen and carbon source*

Ammonium present in the culture medium was determined after 12 h growth. The cultures were grown with shaking at 250 r.p.m., the inoculum had an OD₅₄₀ of 0.1.

Strain	Ammonium excretion	
	(μmol ml ⁻¹)	[μmol (mg protein) ⁻¹]
Wild-type	0.15	8.29
GDH ⁻	0.29	13.18
GS [±]	0.42	18.50
GDH ⁻ ;GS [±]	1.92	22.85

With glutamine as nitrogen and carbon source both the wild-type strain and the GDH⁻;GS[±] mutant strain excreted ammonium as the result of glutamine degradation; however, the double mutant strain excreted almost three times more ammonium than the wild-type (Table 3).

We reported previously (Calderón *et al.*, 1985) that in *N. crassa* glutamine is degraded by the glutamine transaminase- ω -amidase pathway. Table 1 shows that T-Gln activity was present in the wild-type and the double mutant strain grown on glutamine in the presence or absence of

Table 4. *Effect of inhibitors on $^{14}\text{CO}_2$ release from [^{14}C]glutamine by the wild-type and GOGAT⁻ strains growing on glutamine as sole carbon and nitrogen source*

Strain	Inhibitor	$^{14}\text{CO}_2$ release
		[c.p.m. (mg protein) ⁻¹]
Wild-type	None	55 126
	DON (1 mM)	3 626
	AOA (10 mM)	9 831
GOGAT ⁻	None	21 574
	DON (1 mM)	2 079
	AOA (10 mM)	3 753

Table 5. *$^{14}\text{CO}_2$ release by the wild-type, GDH⁻, GS[±] and GDH⁻; GS[±] strains growing on 5 mM-glutamine as sole nitrogen and carbon source*

$^{14}\text{CO}_2$ released was determined during 12 h growth on 5 mM-glutamine and 0.2 μCi (7.4 kBq) ml^{-1} [U- ^{14}C]glutamine. The cultures were grown with shaking at 250 r.p.m.; the inoculum had an OD₅₄₀ of 0.1.

Strain	$^{14}\text{CO}_2$ release	
	c.p.m.	[c.p.m. (mg protein) ⁻¹]
Wild-type	325 371	442 681
GDH ⁻	330 629	430 506
GS [±]	530 834	592 448
GDH ⁻ ; GS [±]	1 331 067	313 930

sucrose; activity was 62% higher in the GDH⁻; GS[±] strain growing on glutamine as nitrogen and carbon source as compared to the wild-type strain (Table 1).

Although low NADP-GDH and GS activities were present in the wild-type strain growing on glutamine without sucrose (Table 1), some of the ammonium released from glutamine must be incorporated since the GDH⁻ and GS[±] single mutant strains excreted 59% and 123%, respectively, more ammonium than the wild-type strain (Table 3) and did not grow as well as the GDH⁻; GS[±] strain under such conditions (Fig. 1a).

Glutamine degradation and synthesis

The glutamine degradation pathways of *N. crassa* growing on glutamine as sole nitrogen and carbon source were demonstrated by incubating the wild-type and GOGAT⁻ strains with [^{14}C]glutamine and measuring CO_2 production (Table 4). In the wild-type strain, DON, an inhibitor of amidotransferase and ω -amidase activities, lowered CO_2 production by 93%; when AOA, an inhibitor of aminotransferases was added, $^{14}\text{CO}_2$ release fell by 82% (Table 3). The amount of $^{14}\text{CO}_2$ released by the GOGAT⁻ strain was only 39% of that released by the wild-type; DON inhibited by 90% and AOA inhibited by 83% the amount of $^{14}\text{CO}_2$ released by this strain (Table 4).

These results indicate that glutamine is degraded to 2-oxoglutarate by GOGAT, the catabolic NAD-GDH and the glutamine transaminase- ω -amidase pathway. Glutamine oxidation was also determined in the single mutants GDH⁻ and GS[±], in the double mutant GDH⁻; GS[±] and in the wild-type strain. The mutant strains oxidized more glutamine than the wild-type strain. The wild-type strain and the single mutant strains oxidized more glutamine per mg of protein than the double mutant strain. However, total CO_2 production was four times greater in the double mutant strain compared to the wild-type strain (Table 5).

Glutamine synthesis in the presence of glutamine as sole nitrogen and carbon source was demonstrated by incubating the wild-type and mutant strains with [^{14}C]glutamate. Glutamine labelling in the wild-type strain was five and thirteen times greater, respectively, than in the GS[±] and GDH⁻; GS[±] strains, in spite of the higher specific radioactivity found in glutamate

Table 6. Incorporation of label from [^{14}C]glutamate into glutamine in the wild-type, $\text{GS}^\pm$ and GDH^- ; $\text{GS}^\pm$ strains growing on 5 mM-glutamine as sole nitrogen and carbon source

Strain	Glutamine labelling [c.p.m. (mg protein) $^{-1}$]	Specific radioactivity found in glutamate [c.p.m. ($\mu\text{mol glutamate}$) $^{-1}$]
Wild-type	37524	230000
$\text{GS}^\pm$	6905	332742
GDH^- ; $\text{GS}^\pm$	2788	343685

(Table 6). This indicates that the ammonium that comes from glutamine degradation is sequentially converted to glutamine. The fourfold lower glutamine content in the GDH^- ; $\text{GS}^\pm$ strain in comparison with the wild-type strain (Table 2) indicates that glutamine cycling is severely impaired in the double mutant.

DISCUSSION

In *N. crassa* glutamine is degraded by the glutamine transaminase- ω -amidase pathway to ammonium and 2-oxoglutarate (Calderón *et al.*, 1985) and the ammonium released by this pathway is assimilated by NADP-GDH and by GS, thus leading to the operation of a glutamine cycle by which this amino acid is continually degraded and resynthesized (Calderón & Mora, 1985). Glutamine is also cycled by GOGAT to glutamate (Calderón & Mora, 1985).

Although glutamine is converted to 2-oxoglutarate, this amino acid cannot be efficiently utilized as a nitrogen and carbon source by wild-type *N. crassa* (Mora *et al.*, 1980), possibly because of the great expenditure of energy as a result of glutamine cycling (Calderón & Mora, 1985). There have been reports indicating that a great amount of energy is expended in glutamine synthesis. It has been shown that when ammonium is added to an ammonium limited culture of *E. coli*, there is a burst of glutamine synthesis which imposes a drastic drain on ATP; the accumulated glutamine activates the enzymes for GS inactivation by adenylation and thus the ATP pool is replenished (Wohlhueter *et al.*, 1973). Fisher *et al.* (1986) reported that in *Bacillus subtilis*, the presence of the herbicide glyphosate results in the largely futile utilization of significant amounts of phosphoenolpyruvate and ATP. The dramatic ability of L-glutamate and L-glutamine to increase glyphosate resistance in *B. subtilis* fits in with a possible consequence of energy drain on nitrogen assimilation.

We used a double mutant lacking NADP-GDH and partially lacking GS in order to prevent the waste of energy and carbon skeleton drain in the synthesis of glutamate and glutamine. This GDH^- ; $\text{GS}^\pm$ double mutant strain grew on glutamine as sole carbon and nitrogen source while the wild-type grew only very slowly. The GDH^- and $\text{GS}^\pm$ single mutants also grew only slightly on glutamine alone, indicating that the synthesis of both glutamate and glutamine contributes to this effect. Better growth of the wild-type strain and the double mutant was obtained in culture conditions of high aeration suggesting that substrate oxidation is a limiting process for growth on glutamine as nitrogen and carbon source. The higher glutamine content of the wild-type strain was proof that the differences in growth between the wild-type and the GDH^- ; $\text{GS}^\pm$ strains were not due to a difference in the uptake of glutamine.

All the enzymes that catabolize glutamine to glutamate, or ammonium and 2-oxoglutarate were present at higher activities in the GDH^- ; $\text{GS}^\pm$ mutant compared to the wild-type when grown on glutamine as nitrogen and carbon source; however, the enzymes that assimilate ammonium were present at higher activity in the wild-type. These differences were reflected in a threefold higher ammonium excretion by the double mutant when compared with the wild-type strain.

Although a higher rate of glutamine oxidation in the GDH^- ; $\text{GS}^\pm$ strain than in the wild-strain might be expected, the higher CO_2 production by the wild-type strain can be explained if the energy from glutamine oxidation is expended on glutamine cycling in the wild-type strain, which cannot happen in the double mutant. The latter is confirmed by the higher excretion of

ammonium on glutamine as nitrogen and carbon source and the decrease of glutamine resynthesis under these conditions in the double mutant. Futile cycles may not be entirely futile since they can be considered as metabolic processes that uncouple catabolism from anabolism and, although they waste energy, they compensate for variations in the carbon supply (Tempest & Neijssell, 1987). In this sense, glutamine cycling is a futile cycle when *N. crassa* is grown on glutamine as sole nitrogen and carbon source but it is necessary in order that glutamine can be utilized as a carbon source (Hernández & Mora, 1986). The differences in growth between the wild-type and the GDH⁻; GS[±] strains can be explained as the result of sparing carbon skeletons and energy when glutamate and glutamine synthesis is severely impaired.

A higher ATP, ADP, NADH and NADPH content was observed in the GDH⁻; GS[±] strain in comparison with the wild-type strain. This suggests that consumption of ATP by GS for glutamine synthesis and NADPH by the NADP-GDH for glutamate synthesis is very high and that a mutant strain which lacks these enzyme activities can save energy which can, in turn, be used for cell growth. Moreover, when growth on ammonium is inhibited by the GS inhibitors glycine or methionine sulfoximine the intracellular concentration of ATP is higher after a short period of incubation, as compared with non-inhibited growth on ammonium (Mora *et al.*, 1988).

On the basis of the results presented here, and taking into account the absence of NADP-GDH and GS activities in the GDH⁻; GS[±] strain, we infer that glutamate that comes from glutamine by the GOGAT reaction can be preferentially degraded through the catabolic NAD-GDH to 2-oxoglutarate since less of this compound is used in glutamate and glutamine synthesis compared with the wild-type strain. In a similar way, the 2-oxoglutarate that comes from the glutamine transaminase- ω -amidase pathway can also be preferentially diverted to the tricarboxylic acid cycle instead of being used to synthesize glutamate. No glutaminase activity is found in *N. crassa* (Calderón *et al.*, 1985, 1989).

Although we found that the biosynthetic NADP-GDH and GS activities were very low in the wild-type strain growing on glutamine as nitrogen and carbon source, the better growth, the higher ammonium excretion and the decrease in glutamine synthesis in the GDH⁻; GS[±] strain indicates that the synthesis of glutamate and glutamine by NADP-GDH and GS is important to spare carbon skeletons and chemical energy.

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REFERENCES

- CALDERÓN, J. & MORA, J. (1985). Glutamine cycling in *Neurospora crassa*. *Journal of General Microbiology* **131**, 3237–3242.
- CALDERÓN, J., MORETT, E. & MORA, J. (1985). The ω -amidase pathway in the degradation of glutamine in *Neurospora crassa*. *Journal of Bacteriology* **161**, 807–809.
- CALDERÓN, J., COOPER, A. J. L., GELBARD, A. S. & MORA, J. (1989). ¹³N Isotope studies of glutamine assimilation pathways in *Neurospora crassa*. *Journal of Bacteriology* **171**, 1772–1774.
- DANTZIG, A. H., WIEGMANN, F. L., JR & NASON, A. (1978). Regulation of glutamate dehydrogenase in nit-2 and am mutants of *Neurospora crassa*. *Journal of Bacteriology* **137**, 1333–1339.
- DÁVILA, G., SÁNCHEZ, F., PALACIOS, R. & MORA, J. (1978). Genetics and physiology of *Neurospora crassa* glutamine auxotrophs. *Journal of Bacteriology* **134**, 693–698.
- DÁVILA, G., LARA, M., GUZMÁN, J. & MORA, J. (1980). Relation between structure and function of *Neurospora crassa* glutamine synthetase. *Biochemical and Biophysical Research Communications* **92**, 134–140.
- ESPÍN, G., PALACIOS, R. & MORA, J. (1979). Glutamine metabolism in nitrogen starved conidia of *Neurospora crassa*. *Journal of General Microbiology* **115**, 59–68.
- FERGUSON, A. R. & SIMS, A. P. (1974). The regulation of glutamine metabolism in *Candida utilis*: the role of glutamine in the control of glutamine synthetase. *Journal of General Microbiology* **80**, 159–171.
- FINCHAM, J. R. S. (1950). Mutant strains on *Neurospora* deficient in aminating ability. *Journal of Biological Chemistry* **181**, 61–73.
- FISCHER, R. S., BERRY, A., GAINES, G. G. & JENSEN, R. A. (1986). Comparative action of glyphosate as a trigger of energy drain in Eubacteria. *Journal of Bacteriology* **168**, 1146–1154.

- HERNÁNDEZ, G. & MORA, J. (1986). Glutamine synthesis regulates sucrose catabolism in *Neurospora crassa*. *Journal of General Microbiology* **132**, 3315–3323.
- HUMMELT, G. & MORA, J. (1980*a*). NADH-dependent glutamate synthase and nitrogen metabolism in *Neurospora crassa*. *Biochemical and Biophysical Research Communications* **92**, 127–133.
- HUMMELT, G. & MORA, J. (1980*b*). Regulation and function of glutamate synthase in *Neurospora crassa*. *Biochemical and Biophysical Research Communications* **96**, 1688–1694.
- LARA, M., BLANCO, L., CAMPOMANES, M., CALVA, E., PALACIOS, R. & MORA, J. (1982). Physiology of ammonium assimilation in *Neurospora crassa*. *Journal of Bacteriology* **150**, 105–112.
- LOMNITZ, A., CALDERÓN, J., HERNÁNDEZ, G. & MORA, J. (1987). Functional analysis of ammonium assimilation enzymes in *Neurospora crassa*. *Journal of General Microbiology* **133**, 2333–2340.
- McKEAG, M. & BROWN, P. R. (1978). Modification of liquid chromatographic nucleotide analysis. *Journal of Chromatography* **152**, 253–254.
- MARZLUF, G. A. (1981). Regulation of nitrogen metabolism and gene expression in fungi. *Microbiological Reviews* **45**, 437–461.
- MORA, J., SALCEDA, R. & SÁNCHEZ, S. (1972). Regulation of arginase activity by intermediates of the arginine biosynthetic pathway in *Neurospora crassa*. *Journal of Bacteriology* **110**, 870–877.
- MORA, Y., CHÁVEZ, O. & MORA, J. (1980). Regulation of *Neurospora crassa* glutamine synthetase by carbon and nitrogen source. *Journal of General Microbiology* **118**, 455–463.
- MORA, J., CALDERÓN, J. & HERNÁNDEZ, G. (1988). Search, assimilation, and turnover of nitrogen in some fungi. In *Nitrogen Control of Metabolic Processes*, chapter 3, pp. 59–81. Edited by S. Sánchez-Esquivel. Boca Raton: CRC Press.
- SÁNCHEZ, F., CAMPOMANES, M., QUINTO, C., HANSBERG, W., MORA, J. & PALACIOS, R. (1978). Nitrogen source regulates glutamine synthetase mRNA levels in *Neurospora crassa*. *Journal of Bacteriology* **136**, 880–885.
- SÁNCHEZ, F., CALVA, E., CAMPOMANES, M., BLANCO, L., GUZMÁN, J., SABORIO, J. L. & PALACIOS, R. (1980). Heterogeneity of glutamine synthetase polypeptides in *Neurospora crassa*. *Journal of Biological Chemistry* **255**, 2231–2234.
- TEMPEST, D. W. & NEISELL, O. M. (1987). Growth yield and energy distribution. In *Escherichia coli and Salmonella typhimurium Cellular and Molecular Biology*, pp. 797–806. Edited by J. L. Neidhardt, K. B. Lowe, B. Magasanik, M. Schaechter & H. E. Umbarger. Washington, DC: American Society for Microbiology.
- VACA, G. & MORA, J. (1977). Nitrogen regulation of arginase in *Neurospora crassa*. *Journal of Bacteriology* **131**, 719–725.
- VICHIDO, I., MORA, Y., QUINTO, C., PALACIOS, R. & MORA, J. (1978). Nitrogen regulation of glutamine synthetase in *Neurospora crassa*. *Journal of General Microbiology* **106**, 251–259.
- VOGEL, H. J. (1964). Distribution of lysine pathways among fungi: evolutionary implications. *American Naturalist* **98**, 435–446.
- WOHLHUETER, R. M., SHUTT, M. M. & HOLZER, H. (1973). Regulation of glutamine synthesis *in vivo* in *Escherichia coli*. In *The Enzymes of Glutamine Metabolism*, pp. 9–64. Edited by S. Prusiner & E. R. Stadtman. New York: Academic Press.