Glutamine Synthesis Regulates Sucrose Catabolism in *Neurospora crassa*

By GEORGINA HERNÁNDEZ AND JAIME MORA*

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

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The effect of glutamine synthesis on sucrose metabolism in *Neurospora crassa* was studied. Different inhibitors of glutamine synthetase were used to inhibit glutamine synthesis in mutants having a low ammonium assimilation capacity. Sucrose utilization was impaired, as indicated by a lower concentration and synthesis of intermediates of the tricarboxylic acid cycle and reduced release of CO₂. We propose that a coordinated regulation of carbon and nitrogen utilization is achieved through sensing of the carbon and nitrogen flows through glutamine synthesis, mediated by changes in the intracellular content of ATP, which is reduced as a consequence of glutamine synthesis.

INTRODUCTION

Carbon and nitrogen metabolism interact in the first instance in the reactions of ammonium assimilation. Carbon compounds, energy and reducing power are required for glutamate and glutamine synthesis. In *Neurospora crassa* glutamate can be synthesized through the participation of two different enzymes, glutamate dehydrogenase (GDH) (EC 1.4.1.4), NADPH dependent, which synthesizes glutamate from 2-oxoglutarate and ammonium (Fincham, 1950; Hernández *et al.*, 1983) and glutamate synthase (GOGAT) (EC 1.4.1.14), NADH dependent, which gives two molecules of glutamate from glutamine and 2-oxoglutarate (Hummelt & Mora, 1980*a, b*). Glutamine synthetase (GS) (EC 6.3.1.2) requires ATP and in *N. crassa* it is found in two different oligomeric forms composed of α and β monomers respectively (Dávila *et al.*, 1980).

Carbon and nitrogen metabolism also interact in the metabolic steps related to the distribution of nitrogen from glutamate and glutamine into several biosynthetic pathways. In *N. crassa* the nitrogen from glutamine is distributed by the enzymes of the ω -amidase pathway as well as by transamidation. Glutamine transaminase synthesizes different amino acids and ω -amidase hydrolyses the resulting 2-oxoglutaminate to 2-oxoglutarate and ammonium (Calderón *et al.*, 1985). The ammonium released by the ω -amidase pathway is assimilated not only by GDH but also by GS, thus leading to the operation of a glutamine cycle in which this amino acid is continually degraded and resynthesized (Calderón *et al.*, 1985; Calderón & Mora, 1985). GOGAT also participates in the distribution of the nitrogen from glutamine (Calderón & Mora, 1985).

The regulation by the nitrogen source of the enzymes that synthesize glutamate or glutamine (Hernández *et al.*, 1983; Vichido *et al.*, 1978; Quinto *et al.*, 1977; Sánchez *et al.*, 1978) and the participation of these enzymes in different ammonium assimilation pathways in *N. crassa* (Lara *et al.*, 1982) have been reported. There have also been reports on the regulation of GS and of GDH by the carbon source in *N. crassa* and other micro-organisms (Ferguson & Sims, 1974*a, b*; Hemmings, 1978; Kapoor & Grover, 1970). Mora *et al.* (1980) reported that when mycelium of

Abbreviations: GDH, glutamate dehydrogenase; GOGAT, glutamate synthase; GS, glutamine synthetase; MS, methionine sulfoximine.

N. crassa was deprived of the carbon source, the GS was degraded; they proposed that this could be a regulatory mechanism to conserve energy. However, despite the cost, in terms of carbon skeletons, reducing power and ATP, of the ammonium assimilation reactions, nothing is known about the effect of glutamine synthesis on carbon metabolism. We therefore looked for an effect of glutamine synthesis on sucrose metabolism in *N. crassa*.

METHODS

Strains. The *Neurospora crassa* wild-type strain 74A, the GDH-deficient mutant *am-1* (GDH⁻) and the GS partial auxotroph *gln-1a* (GS[±]) were from the Fungal Genetics Stock Center (Humboldt State University Foundation, Arcata, Calif., USA). The double mutant *am-1; gln-1a* (GDH⁻; GS[±]) was from the collection of J. Mora.

Growth conditions. Batch cultures of *N. crassa* were grown at 37 °C on Vogel minimal medium (N) (Vogel, 1964) containing 1.5% (w/v) sucrose. At this growth temperature a higher GS activity allowed a higher growth rate in all the strains used and allowed growth of the glutamine auxotroph (GS[±]) on glutamate (Dávila *et al.*, 1978). Other nitrogen sources used in place of or in addition to ammonium nitrate are stated in the text. Growth was measured as described previously (Lara *et al.*, 1982) by total protein concentration determined by the Lowry method.

Determination of GS activity. Cell-free extracts were prepared as previously described (Lara *et al.*, 1982; Vichido *et al.*, 1978). GS activity was measured as described by Ferguson & Sims (1974a). Specific activity was expressed as units (mg total protein)⁻¹, units being $\mu\text{mol } \gamma\text{-glutamyl hydroxamate produced min}^{-1}$.

Ammonium determination. Samples of the medium were collected and ammonium was measured with an Orion electrode (Espín *et al.*, 1979).

Determination of amino acid pools. Samples for amino acid analysis were prepared by homogenizing cells with 80% (v/v) ethanol (Hummelt & Mora, 1980a). The amino acids were separated in an Aminco amino acid analyser, coupled with orthophthalaldehyde (Sigma) and quantified in an Aminco Ratio Fluorometer.

Determination of other metabolites. Mycelial samples were collected on membrane filters (Millipore, type HA, 0.45 μm) and washed with distilled water. Glucose, 2-oxoglutarate and other organic acids were extracted by resuspending the samples in 0.6 M-HClO₄.

Glucose was determined with a glucose kit (Boehringer Mannheim). 2-Oxoglutarate was determined by measuring the initial rate of change in A_{340} , using bovine GDH. A sample of the extract was incubated at room temperature with 30 mM-ammonium sulphate, 0.1 mM-NADH and 0.01 ml of a 2.5% (v/v) solution of bovine liver GDH [50 units (mg protein)⁻¹] (Sigma product no. G2626) in 1 ml final volume of 100 mM-potassium phosphate buffer pH 7.6.

The intracellular concentration of pyruvate (Table 2) was determined by measuring the initial rate of change in A_{340} , using bovine lactic dehydrogenase (LDH). A sample of the extract was incubated at room temperature with 0.1 mM-NADH and 0.01 ml of a 2.5% (v/v) solution of LDH [400 units (mg protein)⁻¹] (Sigma product no. L1006) in 1 ml final volume of 100 mM-Tris buffer pH 7.8.

To measure the CO₂ released from [U-¹⁴C]sucrose, samples of the culture were taken and transferred to a side-arm tube with fresh medium containing 0.06% sucrose. These cultures received a 15 min pulse with [U-¹⁴C]sucrose (0.5 $\mu\text{Ci ml}^{-1}$; 18.5 kBq ml⁻¹). The CO₂ released was collected with NaOH (Mora *et al.*, 1972). The same procedure was followed to determine the CO₂ released from [6-¹⁴C]glucose; in this case the fresh medium contained 0.3% glucose and [6-¹⁴C]glucose (0.5 $\mu\text{Ci ml}^{-1}$; 18.5 kBq ml⁻¹).

Measurement of radioactivity incorporated into 2-oxoglutarate from [U-¹⁴C]sucrose. Cultures were labelled for 15 min with [U-¹⁴C]sucrose at 25 $\mu\text{Ci ml}^{-1}$ in 2 ml medium with the indicated nitrogen source and 0.06% sucrose as carbon source. 2-Oxoglutarate was extracted as described above, and was completely converted to glutamate using 0.1 ml of a 2.5% (v/v) solution of bovine liver GDH [50 units (mg protein)⁻¹] (Sigma; as above) and 60 mM-NADH in 100 mM-potassium phosphate buffer pH 7.6. The reaction was left for 40 min at room temperature and stopped with 80% (v/v) ethanol. The precipitated protein was separated by centrifugation and the supernatant, containing the amino acids and oxo acids, was lyophilized and resuspended in 150 μl lithium citrate buffer (0.30 M-Li⁺, 0.053 M-citrate, pH 2.88) (Benson Co., Reno, Nev., USA; product no. 1216). The amino acids were separated in an Aminco amino acid analyser and the fractions corresponding to glutamate were collected and counted in a liquid scintillation counter. Radioactivity specifically incorporated to 2-oxoglutarate was determined by subtracting the radioactivity incorporated into glutamate in a control without GDH from that incorporated when GDH was added.

Measurement of radioactivity incorporated into tricarboxylic acid cycle intermediates from [U-¹⁴C]sucrose. Cultures were labelled for 15 min with [U-¹⁴C]sucrose at 25 $\mu\text{Ci ml}^{-1}$ in 2 ml medium with the indicated nitrogen source and 0.06% sucrose as the carbon source. The mycelia were collected and the organic acids were extracted as described above. The extracts containing the organic acids were stored at 4 °C before separation and quantification of the tricarboxylic acid cycle intermediates by HPLC (J. Calderón and co-workers, unpublished). The fractions corresponding to the elution time of each tricarboxylic acid cycle intermediate were collected and their radioactivity was measured in a liquid scintillation counter.

Measurement of radioactivity incorporated into amino acids from [U-¹⁴C]sucrose. Cultures were labelled for 15 min with [U-¹⁴C]sucrose at 100 $\mu\text{Ci ml}^{-1}$ in 1 ml medium with the indicated nitrogen source and 0.06% sucrose as the carbon source. The mycelia were collected and the amino acid content was determined as described by Hummelt & Mora (1980a). The radioactivity incorporated into each amino acid was determined in a liquid scintillation counter.

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

RESULTS

Glutamine synthesis and carbon metabolism

The experimental approach used to study whether glutamine synthesis and carbon metabolism were coordinately regulated consisted in inhibiting glutamine synthesis and determining whether sucrose catabolism was also impaired. For this purpose we used mutant strains of *N. crassa* which had a low ammonium assimilation capacity, so as to make it easier to achieve a drastic inhibition of carbon and nitrogen flows.

The GDH⁻ mutant strain has a residual growth on ammonium as nitrogen source (Fincham, 1950) due to the synthesis of glutamate by GOGAT (Hummelt & Mora, 1980a, b). In these circumstances both 2-oxoglutarate [32 nmol (mg protein)⁻¹] and ammonium (Fincham, 1950), substrates of GDH, accumulate intracellularly as a result of the partial block of glutamate synthesis. When glycine or serine is added to the medium, the growth of the GDH⁻ mutant is totally arrested (Fincham, 1950). These amino acids are inhibitors of GS from several sources (Stadtman & Ginsburg, 1974). They are effective inhibitors of *N. crassa* GS activity *in vitro* at low glutamate concentrations and they also inhibit glutamine synthesis *in vivo* (Hernández *et al.*, 1986). We explain the complete blockage of growth on ammonium of the GDH⁻ mutant by glycine and serine as the result of the inhibition of glutamine synthesis *in vivo*. We studied the relation between glutamine synthesis and carbon catabolism from sucrose in this GDH⁻ mutant by inhibiting GS activity with glycine and serine and determining the intracellular pools of 2-oxoglutarate. After 12 h incubation under these conditions the intracellular pools of 2-oxoglutarate in the conidia were very low: 2.8 nmol (mg protein)⁻¹ on ammonium plus glycine and 2.4 nmol (mg protein)⁻¹ on ammonium plus serine. These results indicate an inhibition of sucrose catabolism which may also contribute to the growth inhibition of the GDH⁻ mutant by glycine and serine. The inhibitory effect of these amino acids on growth of conidia of the GDH⁻ mutant strain was reversible, since after 12 h incubation on ammonium plus an inhibitory amino acid the conidia were able to grow on ammonium as the sole nitrogen source (data not shown). To see whether the decreased 2-oxoglutarate content was specifically related to the inhibition of GS by glycine or serine, we tried to inhibit the growth of the GDH⁻ mutant with methionine sulfoximine (MS), a specific inhibitor of GS activity (Ronzio & Meister, 1968). In this condition, the 2-oxoglutarate pool in the conidia was barely detectable [0.9 nmol (mg protein)⁻¹]. The same phenomenon was observed when growth of the wild-type strain on ammonium was inhibited with MS (data not shown). These results suggested that there could be a direct relation between glutamine synthesis and 2-oxoglutarate concentration.

The effect of the inhibition of glutamine synthesis on sucrose catabolism was directly tested by determining the synthesis of tricarboxylic acid cycle intermediates over a short period of time following transfer of previously grown mycelia of the GDH⁻ mutant to ammonium or ammonium plus an inhibitor of GS (glycine or MS) (Table 1). At this time there was a very small difference in growth between each culture and the intracellular glucose content was similar in every condition (data not shown). The specific radioactivity of the tricarboxylic acid cycle intermediates, 2-oxoglutarate, pyruvate, succinate and malate, was lower in mycelium incubated on ammonium plus either glycine or MS; the effect was more apparent in the presence of glycine (Table 1).

These experiments indicate that the inhibition of glutamine synthesis prevents sucrose catabolism at the level of synthesis of the tricarboxylic acid cycle intermediates.

Table 1. *Synthesis of tricarboxylic acid cycle intermediates in vivo in the GDH⁻ mutant*

Cultures were grown for 12 h on glutamate and then transferred to the indicated nitrogen source. The concentrations used were 25 mM-ammonium, 50 mM-glycine and 3 mM-MS. The tricarboxylic acid cycle intermediates synthesized *in vivo* were quantified after 30 min incubation on the indicated nitrogen source; the cultures received a pulse with [U-¹⁴C]sucrose as described in Methods. The experiment was repeated three times; representative results are shown.

Nitrogen source	Specific radioactivity (c.p.m. nmol ⁻¹)			
	2-Oxo-glutarate	Pyruvate	Succinate	Malate
Ammonium	706	252918	13016	419909
Ammonium + glycine	79	28 598	3702	ND
Ammonium + MS	159	115978	10930	130423

ND, Not detected.

Table 2. *Metabolite contents in the GDH⁻;GS[±] mutant*

Conidia were incubated for 12 h with the indicated nitrogen sources. The amino acid concentrations used were 5 mM-glutamine and 50 mM-glycine, serine or leucine. The experiment was repeated twice; representative results are shown.

Nitrogen source	Growth (μg protein ml ⁻¹)	Ammonium released [μmol (mg protein) ⁻¹]	Metabolite content [nmol (mg protein) ⁻¹]				
			Glutamate	Glutamine	2-Oxo-glutarate	Pyruvate	Glucose
Glutamine	321	6	136	ND	27	23	26
Glutamine + glycine	2	23	33	141	3	3	78
Glutamine + serine	0	16	7	422	5	3	85
Glutamine + leucine	77*	1	152	279	15	11	10

ND, Not detected.

*There was optimal growth, after an initial lag phase.

Glutamine recycling and carbon metabolism

Glutamine is a very good nitrogen source in *N. crassa* (Vichido *et al.*, 1978). However, mutants lacking some of the ammonium assimilation related enzyme activities, e.g. GDH⁻, GOGAT⁻;GDH⁻, and GDH⁻;GS[±] strains, have a slower growth rate and/or excrete ammonium into the medium when glutamine is used as nitrogen source (Calderón *et al.*, 1985; Calderón & Mora, 1985). This phenomenon is explained by the reduced capacity of the mutants to assimilate ammonium derived from glutamine degradation, thus indicating recycling of glutamine (Calderón & Mora, 1985).

Arrest of growth on ammonium when glutamine synthesis was inhibited was to be expected; however, as the inhibition of glutamine synthesis also affected carbon catabolism from sucrose (Table 1) this could be a sufficient condition for the inhibition of growth even with glutamine present. The latter assumption was investigated by using the double mutant GDH⁻;GS[±], which has a low synthesis of both glutamate and glutamine, so that maximal inhibition of glutamine synthesis by glycine or serine could be achieved: these amino acids very effectively inhibit the *N. crassa* GS activity *in vitro* when glutamate concentration is low (Hernández *et al.*, 1986).

When glutamine was the sole nitrogen source, the culture grew optimally and excreted ammonium into the medium, and the glutamine pool was undetectable (Table 2). We interpret

Table 3. *Synthesis of tricarboxylic acid cycle intermediates in vivo and CO₂ release in the wild-type and GS[±] mutant strains grown on glutamine*

Cultures were pregrown for 12 h on 5 mM-glutamate and then transferred to 5 mM-glutamine. The tricarboxylic acid cycle intermediates synthesized *in vivo* and CO₂ released from sucrose were quantified after 30 min incubation on glutamine; the cultures received a pulse with [U-¹⁴C]sucrose as described in Methods. The experiment was repeated twice; representative results are shown.

Strain	Specific radioactivity (c.p.m. nmol ⁻¹)				CO ₂ from sucrose [c.p.m. (mg protein) ⁻¹]
	2-Oxo-glutarate	Pyruvate	Succinate	Malate	
Wild-type	1798	75075	19563	3527315	23520
GS [±] mutant	96	16038	1813	69185	9137

the latter as being the result of the degradation of glutamine when the cells were grown on this amino acid as nitrogen source and of the very low capacity of the GDH⁻;GS[±] mutant to reassimilate ammonia and to synthesize glutamine. In the presence of glutamine plus glycine or serine, the growth of the GDH⁻;GS[±] mutant was completely abolished, and the non-growing conidia excreted more ammonium in proportion to their protein content than did the mycelium growing on glutamine alone (Table 2). Under these conditions, a decrease in intracellular glutamate and a large increase in intracellular glutamine were observed (Table 2). The increase in glutamine was probably due to accumulation from the medium after 12 h incubation in the absence of growth.

In this experiment leucine was used as a control because it competes with glutamine uptake in a similar way to the inhibitory amino acids (Pall, 1969) without inhibiting GS activity. In our conditions, glutamine uptake was lower in the presence of either glycine, serine or leucine than with glutamine alone (data not shown). When leucine was added to the medium together with glutamine, the GDH⁻;GS[±] mutant was able to grow after a lag phase; its excretion of ammonium was barely detectable and it had high intracellular pools of glutamate and glutamine (Table 2).

When the GDH⁻;GS[±] mutant was incubated on glutamine plus glycine or serine, the intracellular pools of pyruvate and 2-oxoglutarate in the inhibited conidia were very low as compared to those found in conidia germinating on glutamine alone or on glutamine plus leucine (Table 2). The intracellular pools of glucose were higher in the inhibited conidia incubated on glutamine plus glycine or serine than in those incubated on glutamine plus leucine or glutamine alone (Table 2). This indicates that the low pools of 2-oxoglutarate and pyruvate found on glutamine plus glycine or serine (Table 2) were not due to the lack of glucose. Another observation consistent with 2-oxoglutarate deficiency being responsible for the low glutamate content was the presence of a high activity of a glycine:2-oxoglutarate transaminase (data not shown), which could provide intracellular glutamate whenever glycine and 2-oxoglutarate were present.

The growth-inhibitory effect of glycine and serine on the GDH⁻;GS[±] mutant strain incubated on glutamine was reversible, since the conidia were able to grow if the inhibitors were removed after 12 h.

We conclude that glutamine synthesis was required for growth even in the presence of this amino acid as the nitrogen source, since the inhibitory effect of glycine and serine on growth of the GDH⁻;GS[±] mutant correlated with a deficiency of pyruvate, 2-oxoglutarate and glutamate, and not with glutamine limitation (Table 2).

The data presented in Table 2 lead us to propose that glutamine synthesis, rather than a particular absolute glutamine content, is necessary for optimal utilization of the carbon source, and that glutamine cycling is a necessary condition for growth even in the presence of glutamine. To obtain further support for this proposition, we measured sucrose catabolism in the presence of glutamine in the wild-type strain as compared to the GS[±] mutant, which has a single mutation that alters the structure of the GS β polypeptide (Dávila *et al.*, 1983), has a 20-fold lower GS

Fig. 1. Growth and amino acid pools of the GS^+ mutant. The culture was grown for 12 h on 5 mM-glutamate and then the mycelium was washed and divided into two cultures. One culture (open symbols) was transferred to 5 mM-glutamine, at time 0. The other culture (filled symbols) was first incubated for 1 h in a medium without a nitrogen source and with 0.3 mM-MS, then washed and transferred to 5 mM-glutamine. ○, ●, Growth; △, ▲, intracellular glutamate; □, ■, intracellular glutamine. The experiment was repeated at least three times; representative results are shown.

activity than the wild-type and is able to grow on glutamate at 37 °C (Dávila *et al.*, 1978). The synthesis of tricarboxylic acid cycle intermediates *in vivo* and the CO_2 released from sucrose were measured 30 min after transfer of previously grown mycelia of the two strains from glutamate to glutamine as the nitrogen source, where their growth rate was similar. The specific radioactivity of 2-oxoglutarate, pyruvate, succinate and malate was higher in the wild-type than in the GS^+ mutant strain when grown on glutamine (Table 3). In addition, the CO_2 released from $[U-^{14}C]$ sucrose was lower in the GS^+ mutant, whose synthesis of glutamine is lower. This effect was not due to a dilution of the label by glucose accumulation, since the total intracellular glucose content was 3.6-fold higher in the wild-type than in the GS^+ mutant. The synthesis of glutamate was similar in both strains (data not shown). We propose that when glutamine synthesis is lower due to the partial block on GS activity in the GS^+ mutant strain, carbon catabolism is impaired at the level of the synthesis of intermediates of the tricarboxylic acid cycle, reflected in a diminution of CO_2 released from sucrose (Table 3).

This proposal was further tested under conditions of maximal inhibition of GS activity. This was achieved by adding MS to the GS^+ mutant, thus reducing the GS activity 150-fold as compared to that in the wild-type strain. Since MS is an irreversible inhibitor of GS (Ronzio & Meister, 1968), the GS activity was still inhibited 2 h after removal of the inhibitor (Table 4); activity was regained after 4 h as a result of enzyme synthesis (data not shown). The results of these experiments are shown in Fig. 1 and Table 4. In the presence of MS, no glutamine pool was detected and the glutamate pool increased (Fig. 1). When this culture was shifted to glutamine, it

Table 4. GS activity, synthesis of glutamine, glutamate and 2-oxoglutarate *in vivo* and CO₂ release from glucose in the GS[±] strain

After 12 h growth on 5 mM-glutamate the culture was washed and transferred to 5 mM-glutamine for 2 h (a) or to 0.5 mM-MS for 1 h followed by washing and transfer to 5 mM-glutamine for 2 h (b). All the parameters shown were measured after 2 h growth on glutamine. The experiment was repeated at least three times; representative results are shown.

Condition	GS specific activity [U (mg protein) ⁻¹]	Glutamine from sucrose* (c.p.m. µmol ⁻¹)	Glutamate from sucrose* (c.p.m. µmol ⁻¹)	2-Oxoglutarate from sucrose* [c.p.m. (mg protein) ⁻¹]	CO ₂ from glucose† [c.p.m. (mg protein) ⁻¹]
(a) Gln	0.0030	1225	5650	2124	4593
(b) MS→Gln	0.0004	163	3132	325	1652

* The cultures received a pulse of [U-¹⁴C]sucrose.

† Different cultures received a pulse of [6-¹⁴C]glucose.

had an initial lag phase that was followed by a slower growth despite its high glutamate content and sizeable glutamine pool (Fig. 1) as compared to the non-inhibited culture. Although the glutamine content was only 2-fold lower in the inhibited culture (Fig. 1) the glutamine synthesis varied 7.5-fold. We measured the carbon catabolism of sucrose after the shift to glutamine. The amount of CO₂ released from [6-¹⁴C]glucose was lower when the GS was inhibited and a correlation with a decrease in 2-oxoglutarate synthesis from sucrose was also apparent (Table 4). This was supported by the lower glutamate synthesis observed when GS was inhibited (Table 4).

DISCUSSION

In this work we explored the relation between glutamine synthesis and carbon catabolism from sucrose. By using mutant strains (GDH⁻, GS[±] and the double mutant GDH⁻; GS[±]) with a reduced ability to assimilate ammonium through glutamate, and glutamine and/or GS inhibitors, nitrogen and carbon metabolism were both blocked.

When mycelium of the GDH⁻ mutant was incubated on ammonium plus an inhibitor of GS, sucrose catabolism decreased as shown by a lower synthesis of several tricarboxylic acid cycle intermediates (Table 1). This effect was observed after a short period of time during which growth was not affected. Since both glycine, which inhibits GS activity (Stadtman & Ginsburg, 1979; Hernández *et al.*, 1986), and MS, which is a specific GS inhibitor (Ronzio & Meister, 1968), gave the same effect (Table 1) we propose that the diminution in carbon catabolism was an effect specifically related to the inhibition of glutamine synthesis.

A relevant finding was that the inhibition of glutamine synthesis was sufficient to inhibit growth even in the presence of glutamine. We observed that with the GDH⁻; GS[±] mutant on glutamine plus a GS inhibitory amino acid, growth was completely arrested and the intracellular levels of 2-oxoglutarate and pyruvate were low (Table 2). On glutamine plus leucine, not a GS inhibitor, there was a minor effect on the content of tricarboxylic acid cycle intermediates (Table 2). This can be explained by the lag phase that precedes growth under these conditions, and may also explain the glutamine accumulation (Table 2). In addition, the presence of the inhibitors was not essential since an impairment of sucrose catabolism was also observed in mycelia of a partial glutamine auxotroph (GS[±]) growing on glutamine as the sole nitrogen source (Table 3).

The effect of inhibiting glutamine synthesis was further enhanced when MS, an inhibitor of GS activity, was added to the GS[±] mutant in the presence of glutamine (Table 4, Fig. 1). Under these conditions a higher inhibition of GS activity was observed, with a consequent decrease in synthesis of glutamine, glutamate, and 2-oxoglutarate from sucrose and CO₂ release from [6-¹⁴C]glucose (Table 4). The outcome was a low growth rate in the presence of a sizeable intracellular glutamine pool (Fig. 1).

These data thus support our conclusion that growth inhibition is not due to a lack of glutamine content. We have reported (Hernández *et al.*, 1986) that when growth and GS were inhibited by

glycine in the GS[±] mutant in the presence of glutamate, the synthesis of glutamine *in vivo* was 100-fold lower while the absolute glutamine content was only 2-fold lower as compared to the culture growing on glutamate.

The data presented in this work indicate that in *N. crassa* there is coordinated regulation of carbon and nitrogen source utilization, i.e. when nitrogen assimilation was blocked through the inhibition of glutamine synthesis, carbon catabolism from sucrose decreased. This may explain the necessity for glutamine cycling (Calderón *et al.*, 1985; Calderón & Mora, 1985). The drain of ATP through glutamine synthesis and the regulation of carbon metabolism by energy charge could be considered as a point of interaction in the coordinated regulation of carbon and nitrogen metabolism. An observation consistent with this proposition was that in *N. crassa* the intracellular concentrations of ATP were raised when glutamine synthesis was inhibited (G. Hernández and co-workers, unpublished). It has been reported (Wohlhueter *et al.*, 1973) that when ammonium is added to an ammonium-limited culture of *Escherichia 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. These data suggest that the amount of ATP spent in glutamine synthesis may be important to the cell. The control of glycolysis and respiration by energy charge is well documented (Atkinson, 1977). We propose that whenever glutamine synthesis is blocked the energy status of the cell improves with the consequent inhibition of carbon catabolism. This coordinated regulatory mechanism could allow the cell to avoid the accumulation of carbon skeletons when synthesis of the universal nitrogen donors is blocked.

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