Regulation of Synthesis of Glutamate Dehydrogenase and Glutamine Synthetase in Micro-organisms

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1. *Aspergillus nidulans*, *Neurospora crassa* and *Escherichia coli* were grown on media containing a range of concentrations of nitrate, or ammonia, or urea, or L-glutamate, or L-glutamine as the sole source of nitrogen and the glutamate dehydrogenase and glutamine synthetase of the cells measured. 2. *Aspergillus*, *Neurospora* and *Escherichia coli* cells, grown on L-glutamate or on high concentrations of ammonia or on high concentrations of urea, possessed low glutamate dehydrogenase activity compared with cells grown on other nitrogen sources. 3. *Aspergillus*, *Neurospora* and *Escherichia coli* cells grown on L-glutamate possessed high glutamine synthetase activity compared with cells grown on other nitrogen sources. 4. The hypothesis is proposed that in *Aspergillus*, *Neurospora* and *Escherichia coli* L-glutamate represses the synthesis of glutamate dehydrogenase and L-glutamine represses the synthesis of glutamine synthetase. 5. A comparison of the glutamine-synthesizing activity and the γ -glutamyltransferase activity of glutamine synthetase in *Aspergillus* and *Neurospora* gave no indication that these fungi produce different forms of glutamine synthetase when grown on ammonia or L-glutamate as nitrogen sources.

It has been observed in both fungi and bacteria that the amount and type of nitrogen source available to the cells during growth considerably affect the activities of both glutamate dehydrogenase [L-glutamate-NADP⁺ oxidoreductase (deaminating) (EC 1.4.1.3)] and glutamine synthetase [L-glutamate-ammonia ligase (ADP) (EC 6.3.1.2)]. In *Neurospora crassa* (Sanwal & Lata, 1962) growth on high concentrations of urea, or ammonia, or L-glutamate as sole nitrogen source results in low activities of glutamate dehydrogenase compared with the activities found in cells grown on low concentrations of ammonia. In *Escherichia coli* (Woolfolk, Shapiro & Stadtman, 1966) growth on ammonia results in high activity of glutamate dehydrogenase and low activity of glutamine synthetase, whereas growth on L-glutamate results in low activity of glutamate dehydrogenase and high activity of glutamine synthetase. It has also been reported that growth on ammonia results in low glutamine synthetase activity in yeast (Kohlhaw, Dragert & Holzer, 1965) and that growth on L-glutamine results in low glutamine synthetase activity in *Lactobacillus arabinosus* (Ravel, Humpherys & Shive, 1965) and in *E. coli* (Wu & Yuan, 1968). In fact there are reports, for a variety of micro-

organisms, that urea, ammonia and L-glutamate repress the synthesis of glutamate dehydrogenase and that L-glutamate induces and L-glutamine represses the synthesis of glutamine synthetase. It is difficult on the basis of published data to provide a consistent model to explain the action of these compounds on the synthesis of the two enzymes. Several authors have speculated about indirect effects of these compounds on the general nitrogen status of the cells to account for the activities of glutamate dehydrogenase and glutamine synthetase observed (Woolfolk *et al.* 1966; Wu & Yuan, 1968). The chief objective of the work reported here was to clarify the action of various low-molecular-weight compounds on the induction and repression of glutamate dehydrogenase and glutamine synthetase in micro-organisms.

It has been reported by Kingdon, Shapiro & Stadtman (1967) that *E. coli* produces two distinct forms of glutamine synthetase, depending on the manner in which the organism is grown. The two forms of the enzyme can be distinguished by their catalytic activity. A comparison of certain properties of the glutamine synthetase from *Aspergillus* and *Neurospora* and those reported for glutamine synthetase from *E. coli* is reported here.

MATERIALS AND METHODS

Aspergillus nidulans strains. A biotin-requiring strain bi-1 was used as the prototrophic strain with respect to nitrogen metabolism. The mutant *niaD*-15 lacked nitrate reductase activity; the mutant *niaA*-4 lacked nitrite reductase activity; the mutant *niaB*-1 lacked both nitrate and nitrite reductase activities (Pateman, Rever & Cove, 1967). The mutant UZ-4 lacked urease activity (Scazzocchio & Darlington, 1967).

Neurospora crassa. The prototrophic strain was basically St Lawrence 74A (Pateman, 1959).

Escherichia coli. Stock laboratory cultures of *E. coli* B and *E. coli* K were used.

Cell culture and preparation of cell-free extracts. Mycelium of *Aspergillus* and *Neurospora* was grown in shaken culture for 20 hr. at 25°. The harvesting and preparation of cell-free extracts was essentially as described for *Aspergillus* by Cove (1966). *E. coli* was grown in shaken culture for 20 hr. at 37° in 200 ml. volumes of medium contained in 1 l. Erlenmeyer flasks. The inoculum was 5 ml. of a nutrient broth culture grown overnight to completion. The medium contained (per l.): Na₂HPO₄, 6 g.; KH₂PO₄, 3 g.; NaCl, 3 g.; MgCl₂, 40 mg.; Na₂SO₄, 116 mg.; glucose, 10 g.; made up in glass-distilled water. The nitrogen source is specified in the text. The cells were harvested by pouring the 200 ml. of cell suspension from each growth flask over 100 ml. of crushed frozen 0.1% NaCl soln. in a cold flask. The cold cells were centrifuged at 10000g. for 20 min. at 0°. The supernatant was discarded and the cell pellet frozen and stored in liquid N₂. For extraction frozen cells were ground with glass in a cold mortar in 100 mm-tris-HCl buffer, pH 7.2, plus 1 mM-EDTA. The broken cells were centrifuged at 65000g for 30 min. at 0° and the supernatant was used as the source of enzyme. The quantity of cells used was such that the resultant cell-free extracts contained between 0.5 mg. and 1.6 mg. of soluble protein/ml.

Chemicals. Wherever possible analytical-grade chemicals were used. ATP labelled with ³²P in the γ-position was prepared as described by Glynn & Chappell (1964) and kindly donated by P. C. L. Wong, Flinders University, South Australia.

Enzyme assays. All cell-free extracts were made in 100 mM-tris-HCl buffer, pH 7.2, plus 1 mM-EDTA.

Glutamate dehydrogenase. The reduction of NADP⁺ was measured by the change in *E*₃₄₀ in a recording spectrophotometer. The assay mixture contained: monosodium L-glutamate, 16.9 mg.; NADP⁺, 0.4 mg.; cell-free extract, 10–100 μl.; 0.1 M-tris-HCl buffer, pH 8.5; 4 mM-EDTA to a final volume of 3.0 ml. The initial rate of reaction was determined in the presence and absence of L-glutamate at 35°. Specific activities are expressed as nmoles of substrate transformed/min./mg. of protein.

Glutamine synthetase-γ-glutamyl transfer reaction. The procedure was that given by Woolfolk *et al.* (1966) except that the composition of the reaction mixture was: 0.2 M-L-glutamine; 20 mM-sodium arsenate; 3 mM-MnCl₂; 50 mM-hydroxylamine; 1 mM-ADP; 50–100 μl. of cell-free extract; 20 mM-tris-HCl buffer, pH 8.0, to a final volume of 2.0 ml. The reaction mixture was incubated for 5 min. at 37°, the reaction stopped and colour developed by adding 0.5 ml. of acid FeCl₃ soln. The brown colour due to γ-glutamyl hydroxamate formation in reaction mixtures with and without L-glutamine was measured at 540 nm. Specific

activity is expressed as Δ*E*₅₄₀/min./mg. of protein, since it was not possible to obtain an authentic sample of γ-glutamylhydroxamate.

Glutamine synthetase-L-glutamine synthesis reaction. The reaction catalysed *in vivo* by glutamine synthetase is:

It is possible to assay this activity *in vitro* by measuring the phosphate released. The procedure was to use [³²P]ATP and determine the amount of inorganic ³²P released. The reaction mixture, modified from Woolfolk *et al.* (1966), was: 100 mM-L-glutamic acid; aq. 50 mM-NH₃; 50 mM-MgCl₂; 7.6 mM-[³²P]ATP; 50 μl. of cell-free extract; 100 mM-tris-HCl buffer, pH 7.5, to a final volume of 200 μl. After incubation for 5–10 min. at 35° the reaction was stopped by the addition of 1.0 ml. of 3–5% perchloric acid and cooled in ice. The amount of inorganic ³²P was determined by the method given by Murray & Wong (1967). A control tube with NH₃ omitted was used with each assay to allow for the adenosine triphosphatase activity of the cell-free extracts. It was not possible to use this method for the usual cell-free extracts of *E. coli* owing to their high adenosine triphosphate activity.

Protein determination. The amount of soluble protein in cell-free extracts was determined by a modification of the Folin method described by Lowry, Rosebrough, Farr & Randall (1951).

RESULTS

Glutamate dehydrogenase

Effect of the nitrogen source on glutamate dehydrogenase in Aspergillus and Neurospora. *Aspergillus nidulans* was grown under standard conditions with sodium nitrate, ammonium tartrate, urea, L-glutamate or L-glutamine as sole nitrogen sources at nitrogen concentrations ranging from 5 mM to 100 mM. The cells were harvested and the specific activity of glutamate dehydrogenase was determined. The results are given in Fig. 1; each point represents the mean of at least two completely independent determinations of enzyme activity. Under the growth conditions used 5 mM-nitrogen had a slight limiting effect on growth after 20 hr. incubation; all higher concentrations of nitrogen were non-limiting. It can be seen from Fig. 1 that glutamate dehydrogenase activity rose from about 1250 nmoles/min./mg. of protein on low concentrations of nitrate to about 1500–2000 nmoles/min./mg. of protein at all concentrations above 10 mM-nitrate. Growth on low concentrations of ammonia, urea and L-glutamine resulted in intermediate activities of glutamate dehydrogenase with the activity decreasing as the nitrogen concentration increased. Growth on all concentrations of L-glutamate resulted in very low glutamate dehydrogenase activity. Similar results were obtained with *Neurospora crassa*.

Effect of the nitrogen source on glutamate dehydrogenase in E. coli B and E. coli K. *E. coli* B and *E. coli*

Fig. 1. Glutamate dehydrogenase activities of *A. nidulans* cells grown on various concentrations and types of nitrogen source: $\square$, sodium nitrate; $\blacksquare$, L-glutamate; $\circ$, ammonia; $\bullet$, urea; $\blacktriangle$, L-glutamine.

K were grown under standard conditions with ammonium tartrate, L-glutamate and L-glutamine as sole nitrogen sources at concentrations ranging from 5 mM- to 150 mM-nitrogen. The cells were harvested and the specific activity of glutamate dehydrogenase was determined. The results are given in Fig. 2; each point represents the mean of at least two completely independent determinations of enzyme activity. The two strains *E. coli* B and *E. coli* K show the same type of response to the various substrates although the absolute specific activities are different, particularly with ammonia. *E. coli* B possesses a specific activity of about 200 nmol/min./mg. of protein on 10 mM-ammonia, which then decreases to about 50 on 150 mM-ammonia. *E. coli* K possesses a specific activity of about 450 nmol/min./mg. of protein on 10 mM-ammonia which decreases to about 150 on 150 mM-ammonia. Both strains of *E. coli* show the same trend of decreasing activities of glutamate dehydrogenase as the external concentration of ammonia in the growth medium is increased.

Both *E. coli* B and *E. coli* K possess very low activities of glutamate dehydrogenase when grown on all concentrations of L-glutamate. Both strains also show intermediate amounts of enzyme activity on all concentrations of L-glutamine. It was not possible to grow either of the *E. coli* strains on nitrate under growing conditions comparable with those used for the other nitrogen sources.

Does nitrate induce glutamate dehydrogenase? One explanation of the high activity of glutamate dehydrogenase in cells grown on nitrate is that nitrate induces the synthesis of this enzyme. In some respects this would seem a reasonable situation since nitrate is reduced to ammonia, which is a substrate of glutamate dehydrogenase. Thus the initial substrate nitrate would induce all the enzymes required for the conversion of nitrate into L-glutamic acid. A comparison of normal cells and mutants unable to convert nitrate into ammonia was made with respect to glutamate dehydrogenase activity after growth on media containing both nitrate and L-glutamate, to see whether nitrate itself or some metabolite produced from it is determining the extent of glutamate dehydrogenase synthesis. Several different mutants of *Aspergillus nidulans* were used: *niaD*, which lacks nitrate reductase activity; *nitA*, which lacks nitrite reductase activity; *nitB*, which lacks both nitrate and nitrite reductase activities (Pateman *et al.* 1967). The growth conditions and the resultant glutamate dehydrogenase activities are given in Table 1. In the wild-type growth on 10 mM-L-glutamate alone results in low glutamate dehydrogenase, but the addition of 10 mM-nitrate greatly increases the activity of glutamate dehydrogenase. In all three types of mutants unable to produce ammonia from nitrate the addition of nitrate does not significantly alter the activity of glutamate dehydrogenase from that found after growth on L-glutamate alone. It is clear that in *Aspergillus* nitrate itself does not induce glutamate dehydrogenase.

Fig. 2. Glutamate dehydrogenase activities of *E. coli* B and *E. coli* K cells grown on various types and concentrations of nitrogen source. $\bullet$, *E. coli* B on ammonia; $\circ$, *E. coli* K on ammonia; $\blacksquare$, *E. coli* B on L-glutamate; $\square$, *E. coli* K on L-glutamate; $\blacktriangle$, *E. coli* B on L-glutamine; $\triangle$, *E. coli* K on L-glutamine.

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Does urea repress glutamate dehydrogenase? Since growth on high concentrations of urea results in a low activity of glutamate dehydrogenase the possibility must be considered that urea represses

Table 1. *Glutamate dehydrogenase in the prototroph and various mutants of A. nidulans grown on L-glutamate and on L-glutamate plus nitrate*

A. nidulans was grown on 10 mM-L-glutamate or on 10 mM-L-glutamate plus 10 mM-sodium nitrate as the sole nitrogen sources. The glutamate dehydrogenase activities are given as nmoles of NADP⁺ reduced/min./mg. of protein. The mutants *niaD-15*, *niA-4* and *niB-1* are unable to convert nitrate into ammonia.

Genotype	Glutamate dehydrogenase activity	
	10 mM-L-Glutamate	10 mM-L-Glutamate + 10 mM-sodium nitrate
<i>niaD-15</i>	340	250
<i>niA-4</i>	240	325
<i>niB-1</i>	180	320
Prototroph	275	945

Table 2. *Glutamate dehydrogenase in the prototroph and the UZ-4 mutant of A. nidulans grown on nitrate and on nitrate plus urea*

Mycelium was grown on 50 mM-sodium nitrate plus 5 mM-urea or on 50 mM-sodium nitrate plus 100 mM-urea as the sole nitrogen sources. The glutamate dehydrogenase activities are given as nmoles of NADP⁺ reduced/min./mg. of protein. The mutant UZ-4 is unable to convert urea into ammonia.

Genotype	Glutamate dehydrogenase activity	
	50 mM-NaNO ₃ + 5 mM-urea	50 mM-NaNO ₃ + 100 mM-urea
UZ-4	1230	1510
Prototroph	1675	120

the synthesis of glutamate dehydrogenase. Microorganisms that can utilize urea as a source of nitrogen produce urease, and consequently ammonia from urea. *Aspergillus* can grow on urea and UZ mutants (Scazzocchio & Darlington, 1967), which lack urease activity, are unable to utilize urea. A comparison of wild-type cells and a UZ-4 mutant was made with respect to glutamate dehydrogenase activity after growth on media containing 100 mM-urea and 50 mM-nitrate, in an attempt to show whether urea itself or some metabolite produced from it affects glutamate dehydrogenase synthesis. The growth conditions and resultant glutamate dehydrogenase activities are given in Table 2. In the wild type 100 mM-urea drastically decreases the high activity of glutamate dehydrogenase found on nitrate alone. In the UZ-4 mutant, which is unable to produce ammonia from urea, the presence of 100 mM-urea does not lower the glutamate dehydrogenase activity. It is clear that urea itself does not repress the synthesis of glutamate dehydrogenase in *Aspergillus*.

Fig. 3. Glutamate dehydrogenase and glutamine synthetase activities of *A. nidulans* cells grown on 100 mM-L-glutamate plus various concentrations of sodium nitrate as nitrogen sources. ●, Glutamate dehydrogenase. ■, Glutamine synthetase, assayed by the release of inorganic ³²P from [³²P]ATP.

Fig. 4. Glutamate dehydrogenase and glutamine synthetase activities of *A. nidulans* cells grown on 100 mM-L-glutamate plus various concentrations of ammonium tartrate as nitrogen sources. ●, Glutamate dehydrogenase. ■, Glutamine synthetase, assayed by the release of inorganic ³²P from [³²P]ATP.

Effect of L-glutamate on the synthesis of glutamate dehydrogenase. In *Aspergillus* and *Neurospora* grown on concentrations of L-glutamate ranging from 5 mM to 100 mM the activity of glutamate dehydrogenase was low. *Aspergillus* was also grown on 100 mM-L-glutamate plus a range of concentrations of either nitrate or ammonia and the cells were assayed for glutamate dehydrogenase activity. The results are given in Figs. 3 and 4. The addition of comparatively small amounts of either nitrate or ammonia considerably increases the low glutamate dehydrogenase activity resulting from growth on L-glutamate alone.

Fig. 5. Glutamine synthetase activities of *A. nidulans* cells grown on various types and concentrations of nitrogen sources. $\square$, Sodium nitrate; $\blacksquare$, L-glutamate; $\circ$, ammonia; $\bullet$, urea; $\blacktriangle$, L-glutamine. Glutamine synthetase was assayed by the γ -glutamyl transfer reaction.

Glutamine synthetase

Effect of the nitrogen source on glutamine synthetase in Aspergillus and Neurospora. Samples of the cells, grown on a range of nitrogen sources, which were assayed for glutamate dehydrogenase (see Fig. 1) were also assayed for glutamine synthetase activity. The results for *Aspergillus* are given in Fig. 5. Similar results were obtained with *Neurospora crassa*. Growth on nitrate, or ammonia, or urea, or L-glutamine results in comparable glutamine synthetase activity. The glutamine synthetase activity in cells grown on all concentrations of L-glutamate is about three times that found in cells grown on other nitrogen sources. The simplest explanation of these results is that L-glutamate induces the synthesis of glutamine synthetase. *Aspergillus* and *Neurospora* were also grown on media containing 100 mM-L-glutamate plus a range of other nitrogen sources such as nitrate and ammonia. The glutamine synthetase activities of the cells grown under these conditions are given in Figs. 3 and 4. The addition of nitrate or ammonia at even 5 mM concentration to medium containing 100 mM-L-glutamate resulted in low glutamine synthetase activity similar to that in cells grown on nitrate or ammonia alone.

Effect of the nitrogen source on glutamine synthetase in E. coli B and E. coli K. Samples of the cells, grown on a range of nitrogen sources, which were assayed for glutamate dehydrogenase (see Fig. 2) were also assayed for glutamine synthetase activity. The results are given in Fig. 6. Both *E. coli* B and *E. coli* K possess low glutamine synthetase activities when grown on all concentrations of ammonia, and

Fig. 6. Glutamine synthetase activities of *E. coli* B and *E. coli* K cells grown on various types and concentrations of nitrogen sources. $\bullet$, *E. coli* B on ammonia; $\circ$, *E. coli* K on ammonia; $\blacksquare$, *E. coli* B on L-glutamate; $\square$, *E. coli* K on L-glutamate; $\blacktriangle$, *E. coli* B on L-glutamine; $\triangle$, *E. coli* K on L-glutamine.

both strains possess high glutamine synthetase activities on all concentrations of L-glutamate. Both strains possess high glutamine synthetase activity when grown on low concentrations of L-glutamine, but the enzyme activity decreases as the concentration of L-glutamine in the growth medium increases.

Ratio of glutamine synthetase synthetic activity to γ -glutamyltransferase activity in extracts of Aspergillus and Neurospora. It has been claimed by Kingdon *et al.* (1967) that *E. coli* W produces two distinct forms of glutamine synthetase, depending on the manner in which the cells are grown. Cells grown on ammonium chloride produce mainly glutamine synthetase I whereas cells grown on L-glutamate produce mainly glutamine synthetase II. (Glutamine synthetase II is an adenylylated form of glutamine synthetase I.) The two forms of the enzyme may be distinguished in a number of ways: in particular, glutamine synthetase I is comparatively active in the synthetic reaction and inactive in the γ -glutamyltransferase reaction whereas the reverse is true for glutamine synthetase II (see Table 3). *Aspergillus* and *Neurospora* were grown on media containing either ammonia or L-glutamate as the sole nitrogen source and the cells assayed for glutamine synthetase by using both the assay for the synthetic reaction and the transferase reaction. The results are given in Table 3 together with some published results (Kingdon *et al.* 1967) for comparison. The synthetic reaction/transferase reaction ratio is similar for both *Aspergillus* and *Neurospora* whether grown on ammonia or L-glutamate. The ratio for *E. coli* is 2:4 for cells grown on

Table 3. *Ratio of glutamine synthetase synthetic activity to γ -glutamyltransferase activity in cells grown on ammonia or L-glutamate*

The glutamine synthetase activity/ γ -glutamyltransferase activity ratio is given for prototrophic *A. nidulans* and *N. crassa* grown on 50mm-ammonia or on 100mm-L-glutamate as the sole sources of nitrogen. The ratio for *E. coli* W grown on ammonia or on L-glutamate is taken from Kingdon *et al.* (1967).

Organism	Glutamine synthetase/ γ -glutamyltransferase	
	Ammonia	L-Glutamate
<i>A. nidulans</i>	1.49	1.18
<i>N. crassa</i>	1.14	1.13
<i>E. coli</i> W	2.40	0.28

ammonia and 0.28 for cells grown on L-glutamate. Thus, unlike *E. coli* W, *Aspergillus* and *Neurospora* do not produce two forms of glutamine synthetase with different activities in the synthetic and transferase reactions when grown on ammonia and L-glutamate.

DISCUSSION

Glutamate dehydrogenase and glutamine synthetase activity in both the fungal and bacterial cells was dependent on the nitrogen source on which the cells were grown.

It is clear that in *Aspergillus* nitrate does not induce and urea does not repress synthesis of glutamate dehydrogenase. Although the same direct proofs through the use of appropriate mutants are not available, it is probable that the situation is the same in *Neurospora* as that in the closely related *Aspergillus*. The apparent effects of nitrate and urea on the synthesis of glutamate dehydrogenase can be attributed to the production of ammonia from them as the first stage in their utilization (see below for more detailed explanation). The simplest hypothesis is that the concentration of L-glutamate alone determines the rate of synthesis of glutamate dehydrogenase. The apparent effects of nitrate, ammonia and urea are due to indirect effects on the concentration of L-glutamate. A high concentration of ammonia results in the production of sufficient L-glutamate to repress the synthesis of glutamate dehydrogenase to the minimum. At lower concentrations of ammonia the effective concentration of L-glutamate is low owing to the utilization of ammonia and L-glutamate by various metabolic reactions, particularly L-glutamine synthesis. The role of nitrate and urea is through their effect on the concentration of ammonia and subsequently on that of L-glutamate. With nitrate it has been shown

in *Aspergillus* (Pateman & Cove, 1967) that the enzymes that reduce nitrate to ammonia are induced by nitrate and repressed by ammonia. The repression by ammonia is more powerful than the induction by nitrate. Thus in cells grown on nitrate alone the internal concentration of ammonia is low and consequently the synthesis of glutamate dehydrogenase is de-repressed. Although not as much is known about the control of nitrate reduction to ammonia in *Neurospora* it is reasonable to suppose that a similar situation is responsible for the high activity of glutamate dehydrogenase when the organism is grown on nitrate. Unfortunately it has not proved possible to grow either of the *E. coli* strains under comparable conditions with nitrate as the sole nitrogen source. With urea the situation is similar in *Aspergillus* to that of cells grown on ammonia. When cells are grown on low concentrations of urea ammonia is produced by the action of urease, but the concentration of ammonia does not result in over-production of L-glutamate and repression of glutamate dehydrogenase. When cells are grown on high concentrations of urea, sufficient ammonia is produced to cause repression of glutamate dehydrogenase. The effect of high concentrations of urea is in direct contrast to that of high concentration of nitrate. Although the synthesis of urease in *Aspergillus* is repressed by ammonia the maximum degree of repression only decreases the urease by about one half (Scazzocchio & Darlington, 1967). Consequently high external concentrations of urea can result in high internal concentrations of ammonia and subsequent repression of glutamate dehydrogenase.

When *Aspergillus* was grown on 100mm-L-glutamate the activity of glutamate dehydrogenase was very low. The addition of comparatively small concentrations of either nitrate or ammonia resulted in two- to three-fold increases in glutamate dehydrogenase. The probable explanation is that ammonia in the cells effectively decreased the internal concentration of L-glutamate as a result of glutamine synthetase activity. The decrease in L-glutamate concentration would result in the partial de-repression of glutamate dehydrogenase synthesis.

The glutamine synthetase activity was similar in *Aspergillus* cells grown on nitrate or ammonia or urea or L-glutamine, but it was increased three- to five-fold in the cells grown on L-glutamate. A possible explanation is that L-glutamate induces the synthesis of glutamine synthetase. The main objection to this hypothesis is that it does not readily account for the low glutamine synthetase activity in the cells grown on 100mm-L-glutamate plus some other nitrogen source at 5mm concentration. Although the addition of a second nitrogen source, particularly ammonia, might result in some extra

utilization of L-glutamate it could not appreciably lower the very high concentration of L-glutamate. Yet the glutamine synthetase activities are low in spite of the presence of a high concentration of the supposed inducer L-glutamate. An alternative hypothesis is that as a result of repression by L-glutamine the concentration of L-glutamine determines the rate of synthesis of glutamine synthetase. On this basis cells utilizing nitrate or ammonia or urea are synthesizing sufficient L-glutamine to keep the synthesis of glutamine synthetase down to the basal amount since neither ammonia nor L-glutamate is limiting. The situation for cells growing on L-glutamate is different. Repression by the high concentration of L-glutamate results in a very low glutamate dehydrogenase activity, yet deamination by the action of glutamate dehydrogenase is the only direct means of obtaining ammonia from L-glutamate available to the cells. Consequently in cells growing on L-glutamate the concentration of ammonia is low and this limits the synthesis of L-glutamine by glutamine synthetase. The low concentration of L-glutamine results in de-repression of the synthesis of glutamine synthetase and consequent high activity of the enzyme. This hypothesis readily explains the low activity of glutamine synthetase found in cells growing on 100mM-L-glutamate plus a second nitrogen source at a much lower concentration. The minor nitrogen source results in the production of ammonia, which together with the L-glutamate allows the production of L-glutamine and consequent repression of glutamine synthetase synthesis.

The arguments presented above support the simple hypothesis that the synthesis of glutamate dehydrogenase is controlled by L-glutamate repression and the synthesis of glutamine synthetase by L-glutamine repression. Most of the relevant data have been obtained from *A. nidulans* but the situa-

tion is almost certainly the same in *N. crassa*. The position is more complex in *E. coli* owing to the presence of two forms of glutamine synthetase, but the hypothesis certainly accounts for the relevant observations reported in the literature and those given here.

Now that the true effectors, L-glutamate and L-glutamine, are known for glutamate dehydrogenase and glutamine synthetase in *A. nidulans* it is possible to devise experiments for the production of mutants that are de-repressed with respect to the synthesis of these enzymes.

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