ABSENCE OF INVOLVEMENT OF GLUTAMINE SYNTHETASE AND OF NAD-LINKED GLUTAMATE DEHYDROGENASE IN THE NITROGEN CATABOLITE REPRESSION OF ARGINASE AND OTHER ENZYMES IN SACCHAROMYCES CEREVISIAE.

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SUMMARY

The escape of several enzymes from "ammonia catabolite repression" in gdhA⁻ (NADP-linked glutamate-dehydrogenase-less) mutants, as well as in gdhCR mutants of Saccharomyces cerevisiae, does not involve glutamine synthetase, either as a positive or as a negative control element. A glutamine-synthetase-less mutant (gln⁻) was used in this demonstration.

In addition to its derepressing effect on the NAD-linked glutamate dehydrogenase, the gdhCR mutation releases "nitrogen catabolite repression" on arginase and allantoinase, as well as glutamine repression on glutamine synthetase. A gdhCS mutation was used to demonstrate that these effects are not mediated through the NAD-linked glutamate dehydrogenase.

The NADP-linked glutamate dehydrogenase (NADP-GDHase) appears to be an important element in the regulation of nitrogen metabolism in Saccharomyces cerevisiae (1-3) as well as in other fungi (4-6). The main support of this idea is the fact that, whereas a strong decrease in activity of some enzymes and uptake systems using nitrogen compounds is observed in cells grown in the presence of NH_4^+, this "ammonia effect"

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is largely released in gdhA$^-$ mutants (which have lost the activity of NADP-GDHase). Whether the NADP-GDHase is involved in this regulation through its catalytic activity or rather more directly as a regulatory molecule (such as a repressor or an inhibitory binding protein) is not completely clarified as yet. However, in the case of Saccharomyces cerevisiae, the second possibility is favoured by the presently available data (3).

On the other hand, a key role of glutamine synthetase in ammonium repression in bacteria was proposed by Streicher, Gurney and Valentine in their remarkable paper on the nitrogen fixation genes (7). A number of data favouring this idea have been obtained (8,9). Recently, a convincing direct demonstration has been presented by Tyler, Deleo and Magasanik (10) showing that the glutamine-synthetase molecule, in its non-adenylated form, is able to activate the transcription of the hut (histidine utilization) operon DNA of Salmonella typhimurium.

In view of these data, it seemed crucial to us to answer the following questions. First, is the effect caused by the lack of NADP-GDHase in the gdhA$^-$ mutants of Saccharomyces cerevisiae mediated through glutamine synthetase ? Secondly, can a high level of glutamine synthetase have the same effect as the lack of NADP-GDHase ? This investigation led us to obtain additional information about the regulatory behaviour of other enzymes involved in nitrogen catabolism.

MATERIALS AND METHODS

Strains. All the strains used are isogenic to the wild-type strain Σ1278b of Saccharomyces cerevisiae. The isolation and characteristics of the following mutations have been described : gdhA$^-$, affecting the structural gene of the NADP-linked glutamate dehydrogenase (1, 11), gdhCR, allelic to ure-2 (11,12), gdhCS, allelic to ng13 (11, 12). The gln$^-$ mutation of strain MG935 has been obtained after ethyl-methanesulfonate mutagenesis. This mutation provokes a specific requirement for

glutamine, which cannot be replaced by NH_4^+, glutamate, NH_4^+ + glutamate, asparagine, and all other substances tested (aspartate, proline, ornithine, citrulline, arginine). It can be seen in the Table of the present paper that no glutamine synthetase activity can be detected in a gln^- strain even in the presence of a gdhCR mutation which is able to dramatically increase this enzyme activity in other strains (Table 1, experiment 18 compared to 11).

Medium and growth conditions have been described (13); medium M was used throughout, but the nitrogen source, which is NH_4^+ in this medium, was replaced in some experiments by either glutamate, glutamine, or NH_4^+ + glutamate, as indicated in Table 1. These amino acids were added to a final concentration of 1 mg per ml; the NH_4^+ concentration remains 0.02 M.

Enzyme essays were performed on French press extracts of exponentially growing cells. The debris are centrifuged and the supernatant dialyzed on Sephadex G-25 (Pharmacia). Enzyme activities are measured as described in the following references : arginase (14), allantoinase (15), NAD- and NADP-linked glutamate dehydrogenases (16), and glutamine synthetase (synthetase assay) (17, 18).

RESULTS

Glutamine synthetase activity in $gdhA^-$ mutants :

It can be seen in Table 1 that the glutamine-synthetase activity is not changed in a $gdhA^-$ mutant under conditions where the arginase and the allantoinase activities are increased compared to the wild-type strain (experiments 6 compared to 2, and 7, to 4). In addition, in the wild-type strain grown on NH_4^+ (experiment 1), a low level of arginase is associated to a relatively high level of glutamine synthetase.

On the other hand, the absence of glutamine-synthetase activity, due to a gln^- mutation, does not prevent the $gdhA^-$ mutation to release the ammonia effect on arginase synthesis (experiment 17 versus 7).

TABLE 1: Enzyme activities(x) in the wild-type strain and in mutants.

Strains and Nitrogen source in growth medium Exp. n°	Arginase	Allantoinase	NAD-GDHase	NADP-GDHase	Glutamine synthetase
Σ1278b (wild type)					
1. NH_4^+	6	0,7	1,2	45-50	4,5-5,1
2. NH_4^+ + glutamate	8	0,35	1	37	2,9
3. glutamate	50	1,9	47	35	14,4-15,8
4. glutamine	10-12		0,9		<0,1
4324c ($gdhA_1^-$)					
5. NH_4^+	40		2	<0,1	
6. NH_4^+ + glutamate	35	3,5	1,3		3
7. glutamine	29				<0,1
12.597a (gdhCR)					
8. NH_4^+	60-80		133-153	19-22	4,7-5,6
9. NH_4^+ + glutamate	95	6,6			
10. glutamate	122		185		
11. glutamine	140		80	17	5
MG935 (gln^-)					
12. glutamine	9		0,7	18	<0,1
11.412d (gdhCS)					
13. NH_4^+	6		0,5		4,3
14. glutamate	50		0,25		15
12.759a ($gdhA_1^-$, gdhCR)					
15. NH_4^+					4
16. NH_4^+ + glutamate	89				5,6
11.064a ($gdhA_1^-$, gln^-)					
17. glutamine	38				
13.836a (gdhCR, gln^-)					
18. glutamine	75		165		<0,1
12.781a (gdhCR, gdhCS)					
19. NH_4^+	53		0,7	24	
20. glutamine					5,4

(x) Enzyme activities in μmoles of product formed per mg of protein per hour at 30°C.

Can a high level of glutamine synthetase have the same effect as the lack of NADP-GDHase ?

It was observed previously that in gdhCR mutants grown on NH_4^+ as nitrogen source the NAD-GDHase activity is more than hundredfold higher than in the wild-type strain, while the NADP-GDHase level is reduced by a factor of two (10, 11; see also experiment 8 compared to 1). The data presented in Table 1 show that the activity of glutamine synthetase in cells grown on glutamine as sole nitrogen source is very low in the wild-type strain (experiment 4), whereas it is at least 50-fold higher in the gdhCR mutant (experiment 11). Under the same growth conditions, the arginase activity in the gdhCR mutant is very high (experiment 11).

If the glutamine-synthetase derepression were responsible for the effect on arginase, this effect should be suppressed when the glutamine-synthetase activity is removed by a gln^- mutation. However, this is not the case (experiment 18).

As a control, the level of arginase was measured in a single gln^- mutant (MG935) grown on glutamine (experiment 12).

It is also striking that the glutamine-synthetase activity of cells grown on NH_4^+ is the same in the gdhCR mutant (experiment 8) as in the wild-type strain (experiment 1), whereas the arginase activity is tenfold higher in the gdhCR mutant. The arginase and the allantoinase activities are also high in the gdhCR mutant grown on NH_4 + glutamate (experiment 9 compared to experiment 2). They are even higher than in the $gdhA^-$ mutant (experiment 6).

Effect of a gln^- mutation on the level of NAD-GDHase activity in a gdhCR mutant :

It can be seen in Table 1 (experiment 18 compared to 11 and 4) that the introduction of a gln^- mutation in a gdhCR mutant does not reduce the high level of NAD-GDHase, showing that the effect of the gdhCR mutation is

not mediated through glutamine synthetase.

Effect of a gdhCS mutation on the level of glutamine synthetase in a gdhCR mutant :

The gdhCS mutation provokes a strong decrease in the level of NAD-GDHase in a gdhCR mutant grown on NH_4^+ (10, 11; see also experiment 19 compared to 8). However, this mutation does not lower the level of glutamine synthetase (experiment 20, compared to 11) and of arginase (experiment 19 compared to 8). This shows that the effect of the gdhCR mutation on these two enzymes is not mediated through the NAD-GDHase.

DISCUSSION AND CONCLUSIONS.

It seems clear that the escape of several enzymes from "ammonia catabolite repression" in $gdhA^-$ as well as in gdhCR mutants of Saccharomyces cerevisiae does not involve glutamine synthetase, either as a positive or as a negative control element. The most direct evidence for this conclusion is the absence of effect of the gln^- mutation either alone or in combination with a $gdhA^-$ or a gdhCR mutation.

The central role of key enzymes of ammonia assimilation in the regulation of nitrogen catabolic systems results in a very efficient integration of nitrogen metabolism. That the enzyme endowed with regulatory properties is the NADP-linked glutamate dehydrogenase in fungi and glutamine synthetase in bacteria is in keeping with the difference in the mechanism of ammonia assimilation which operates in these two groups of organisms.

The fact that a high level of both NAD-GDHase and arginase is observed in gdhCR mutants under conditions where these levels are low in the wild-type strain might be taken as an indication that the NAD-linked glutamate dehydrogenase could activate arginase synthesis. This possibility can be ruled out since, in a double (gdhCR, gdhCS) mutant grown on NH_4^+, the activity of arginase is still high, whereas the level of NAD-GDHase is negligible.

Since the NADP-GDHase activity is reduced by a factor of two in a gdhCR mutant compared to the wild-type strain (10, 11, and this paper), it might be asked whether the effects of the gdhCR mutation are consequences of this lowering of NADP-GDHase activity. This is excluded in the case of NAD-GDHase and glutamine synthetase, since the gdhA$^-$ mutations do not relieve these two enzymes from "ammonia repression" (Table 1, experiments 6,7, compared to 2 and 4). On the basis of all the data obtained until now (3), the reduction in activity of the NADP-GDHase in the gdhCR mutant (19-22 units instead of 45-50 units) is too small to produce any derepression of arginase. The situation is likely to be the same for allantoinase.

The fact that the gdhCR mutation affects not only those enzymes which are also liberated from "ammonia repression" by the gdhA$^-$ mutation, but all the enzymes sensitive to nitrogen regulation which were tested points to the existence of a common element in this regulation. The data presented allow to add that this regulator is none of the enzymes studied here. But the gdhCR mutation might affect a gene coding for a general "nitrogen repressor".

A serious discussion and comparison of our data with those obtained on the regulation of glutamine synthetase in Candida utilis (17-19) is not possible in the present state of our investigation, although some differences are perceptible and interesting problems are raised.

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