RELEASE OF THE "AMMONIA EFFECT" ON THREE CATABOLIC ENZYMES BY NADP-SPECIFIC GLUTAMATE DEHYDROGENASELESS MUTATIONS IN SACCHAROMYCES CEREVISIAE

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SUMMARY

Mutations which inactivate the NADP-glutamate dehydrogenase (anabolic GDHase) pleiotropically release the ammonia inhibition (NH_4^+ effect) on a number of distinct catabolic activities. In addition to releasing inhibition on several permeability functions (1), these mutations suppress the NH_4^+ effect on the synthesis of arginase, urea amidolyase and allantoinase. They do not affect the NH_4^+ effect on the NAD-glutamate dehydrogenase.

Two mechanisms of action of these mutations have to be considered, namely a modification of the process of induction (such as removal of inducer exclusion) and a suppression of nitrogen catabolite repression.

It has been shown recently that NADP-specific glutamate dehydrogenaseless mutants (gdhA$^-$) have lost the NH_4^+ inhibition exerted on the general amino acid permease of Saccharomyces cerevisiae (catabolic permease) as well as on other permeability functions (1).

NH_4^+ strongly affects arginase synthesis (2) and it has been mentioned that a gdhA$^-$ mutation also releases the NH_4^+ effect in this case (1). This is developed here. In addition, it is shown that this mutation extends its effects on two other nitrogen catabolic enzymes.

MATERIALS AND METHODS

Strains. Σ1278b is a wild-type. All strains used are isogenic with Σ1278b except for the additional mutation. 4324c is a segregant with the gdhA$^-$1 mutation (1). The gdhA$^-$2 mutation was obtained in the

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absence of selective pressure for the loss of the NH_4^+ effect ; it is present in strain MG1749.

Medium and growth conditions have been described (3, 4). Medium 150 is devoided of nitrogen nutrient ; if not otherwise mentioned it is supplemented with 3 % glucose, vitamins and a particular nitrogen source, and designated by M. followed by the name of the nitrogen nutrient. M.am. contains 0.02 M. NH_4^+ ; other additions are 1 mg/ml. M.arg. contains 1 mg of L-arginine as nitrogen nutrient ; M.am.+arg. contains 1 mg L-arginine per ml of M.am.,etc...

Enzyme assays are 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 (5), α-glucosidase (6), NAD- and NADP-glutamate dehydrogenase (7), urea amidolyase (8, except that CO_2 was captured by hyamine - 40 µl on filter paper - in the central cup of Warburg cells), allantoinase (9), serine dehydratase (10), threonine dehydratase (11, except that threonine is 4 X 10^{-2}M).

RESULTS

The main result is expressed in experiment 17 of Table 1, to be compared with experiment 3. In these experiments gdhA$^-$ mutants grow at the same rate as wild-type cells because of the presence of glutamate in the medium. The gdhA$^-$ mutation provokes a derepression of the three enzymes arginase, urea amidolyase and allantoinase : their activities increase by a factor of 4, 22 and 10 respectively.

Growth with other nitrogen nutrients (experiments 8 to 14), in the absence of NH_4^+ , or growth in a chemostat limited by NH_4^+ (0.001 M. NH_4^+) (experiment 15) produce the same effect as the gdhA$^-$ mutation. Under these conditions the gdhA$^-$ mutation does not produce any additional increase in arginase activity (experiment 21 versus 13).

The highest level of arginase is obtained by a combination of the release of NH_4^+ effect produced by the gdhA$^-$ mutation, and arginine induction (experiment 19). This high level is independent of the presence of NH_4^+ (experiments 19 and 20). In this particular case, the gdhA$^-$ mutation is likely to act both by releasing nitrogen catabolite repression and by preventing inducer exclusion by NH_4^+ at the level of uptake ; wo do not know what is the contribution of these two factors.

TABLE 1 : Enzyme activities(*) in the wild-type strain and in NADP-glutamate dehydrogenaseless mutants (gdhA⁻).

Strains and growth medium Exp. n°	Gen. time in min.	arginase	urea amido-3 lyase x 10	allantoinase	NAD-glutamate dehydrogenase	serine dehydratase	threonine dehydratase	α-glucosidase
Σ1278b (wild-type)								
1 M.am.	120	6	1		1.2	2	31	0.4
2 M.am. (galactose replaces glucose)		7.5						10
3 M.am.+glutamate	120	8	1	0.35	1			
4 M.am.+arginine	120	20						
5 M.am.+threonine							30	
6 M.am.+urea	120		22					
7 M.am.+serine						2		
8 M.arginine	150	250						
9 M.urea	180		170					
10 M.proline	180	20						
11 M.serine	165	20				2		
12 M.glutamate	150	50	25	1.9	44			
13 M.valine	180	63						0.4
14 M.threonine							54	
15 Chemostat am.limited	170	35						
4324c (gdhA⁻1)								
16 M.am.	210	40						
17 M.am.+glutamate	120	35	22	3.5	1.3	2.4	31	0.4
18 M.am.+urea			230					
19 M.am.+arginine		350						
20 M.arginine		342						
21 M.valine	180	65						
22 M.glutamate		60			41			
23 M.proline		23						
MG1749 (gdhA⁻2)								
24 M.am.+glutamate		52						

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

It is striking that the NAD-glutamate dehydrogenase, the most typical and general catabolic enzyme of nitrogen metabolism in yeast, is not affected by the gdhA$^-$ mutation, in spite of being subject to a strong NH_4^+ effect (12) also present in our strain (experiments 12 and 22 versus 3 and 17). Obviously, that enzyme belongs to another regulatory circuit. In our strain, L-serine- and L-threonine dehydratase have no or a low NH_4^+ effect, and are not affected by the gdhA$^-$ mutation.

α-glucosidase was chosen as a control : this typical enzyme sensitive to glucose effect (13) is also glucose sensitive in our strain, since its activity shows a 40-fold increase when glucose is replaced by galactose (experiment 2 versus 1). It is significant that the gdhA$^-$ mutation does not affect its activity (experiment 17).

DISCUSSION

Since the discovery of two distinct (NAD- and NADP-) glutamate dehydrogenases in yeast (14) and in Neurospora (15), the function of the two enzymes appeared clearly. The am$^-$ mutation in Neurospora reduces the rate of growth on NH_4^+ as sole nitrogen nutrient and this mutation concerns the NADP-specific glutamate dehydrogenase (16). The gdhA$^-$ mutation in S. cerevisiae is of the same type (1). In both cases addition of glutamate restores normal rate of growth. Hence the NADP enzyme can be considered as an anabolic enzyme. The presence of NH_4^+ in a culture medium strongly reduces the synthesis of NAD-glutamate dehydrogenase and this enzyme appears to be a catabolic enzyme which degrades glutamate to provide the cell with NH_4^+ needed at least to form glutamine, one of the most general donors of nitrogen.

The results obtained in S. cerevisiae and presented here show that NADP-glutamate dehydrogenase belongs to the regulatory circuit through which NH_4^+ exerts its action on a number of enzymes : arginase, urea amidolyase, allantoinase.

The gdhA$^-$ mutation has no action on the NH_4^+ effect on the NAD-(catabolic) glutamate dehydrogenase indicating that another mechanism must be involved in that case.

In Aspergillus nidulans gdhA$^-$ mutation has been shown to suppress the NH_4^+ effect on nitrate reductase and xanthine dehydrogenase (17).

Differences in NH_4^+ effect between different organisms is well known in several cases, e.g. between Salmonella typhimurium and

Klebsiella aerogenes (18), or among different wild-type strains of S. cerevisiae (3), the general amino acid permease of which is, as a rule, much less sensitive to NH_4^+ than that of Σ1278b (unpublished). In the same line, L-threonine dehydratase shows a weaker or no NH_4^+ effect in Σ1278b although it does in other strains (19). The results presented here on allantoinase synthesis are difficult to compare with those obtained in Saccharomyces carlsbergensis (20) because of differences in methodology. Using cells under starvation or any condition of unbalanced growth may introduce uncontrollable variables.

Regarding the mechanism of the NH_4^+ effect, our data provide indications in two lines which must be developed further.

1. As was clearly pointed out in the case of the "glucose effect" in bacteria (see 21), since catabolite repression affects inducible enzymes, it is essential to distinguish inducer exclusion at the level of uptake from a true catabolite repression at a level of gene expression. Furthermore, even in the absence of external inducer, the danger remains that reductions in growth rate might produce or increase endogeneous induction.

The fact that gdhA$^-$ mutations promote enzyme synthesis without modification of growth rate (in M.am.+glutamate) and in the absence of inducer strongly suggests a NH_4^+ effect at the level of nitrogen catabolite repression. A definite proof of this will be presented elsewhere, using two types of mutants which have lost the process of induction and show the effect of gdhA$^-$ mutation independently of induction (22).

2. What was reported before (1) and here opens an analysis of NH_4^+ effect. As mentioned, NH_4^+ itself does not act when the NADP-GDHase is not active. As addition of glutamate restores a normal pool of glutamate and amino acids without restoring the NH_4^+ effect, these amino acids taken alone are not the effectors. We shall analyze elsewhere the possible effect of other components of the NADP-glutamate dehydrogenase system (22).

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