GLUTAMINE AND AMMONIA IN NITROGEN CATABOLITE REPRESSION OF SACCHAROMYCES CEREVISIAE

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Summary

The nitrogen catabolite repression of catabolic (NAD) glutamate dehydrogenase (GDHaseC), allantoinase and urea amido-lyase is relieved in gln^-,$gnrR^-$double mutants. The gln^- mutation leads to glutamine auxotrophy; alone it is devoid of regulatory effect. The new $gnrR^-$ mutation alone is detectable by a partial derepression of allantoinase only.

Considering these facts together with the derepressive effect of the $gdhA^-$ mutation on allantoinase, urea amido-lyase and arginase (1), and the absence of gln^-,$gnrR^-$ effect on arginase, one may define three types of nitrogen catabolic enzymes.

1°) arginase, submitted to NH_4^+ effect relieved in a $gdhA^-$ mutant.
2°) GDHaseC, derepressed by the gln^-,$gnrR^-$ combination.
3°) allantoinase and urea amido-lyase under both $gdhA^-$ and gln^-,$gnrR^-$ derepressive actions.

It is suggested that the gln-gnrR system transmits a signal distinct from NH_4^+ such as glutamine or a close derivative.

INTRODUCTION

The syntheses of arginase, of urea amido-lyase, of allantoinase and of the catabolic (NAD) glutamate dehydrogenase (GDHaseC) of S. cerevisiae are subject to nitrogen catabolite repression (NCR) (ref. in 1). Cells are repressed when grown in NH_4^+, glutamine or asparagine as sources of nitrogen. Derepression occurs with glutamate as only nitrogen nutrient as well as in a chemostat with limiting nitrogen nutrient (2) or in gdhCR mutants (3). Arginase has been studied in detail. Non inducible or constitutive operator mutations allow in this case to differentiate induction and nitrogen catabolite repression from occasional indirect effects (2). $gdhA^-$ mutations located in the structural gene for the anabolic (NADP) glutamate dehydrogenase (GDHaseA) (4) release NCR of arginase even in the presence of glutamate or glutamine added to compensate for the loss of the catalytic activity of GDHaseA. The derepression state caused by the $gdhA^-$ mutation on arginase is quantitatively similar to the physiological derepression mentioned above (2). It has been proposed that NH_4^+ is the metabolic signal received by the (NADP) glutamate dehydrogenase which

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transmits the signal to the gene with the participation of a molecule GDHCR issued from the gene gdhCR (3).

The GDHaseC is not derepressed in gdhA$^-$ mutants (1) ; this enzyme is under the control of a distinct regulatory mechanism. A first question is the nature of the input signal. NH_4^+, glutamine and asparagine are equivalent for GDHaseC and arginase repression. In Aspergillus nidulans this is not so for a number of enzymes and Hynes opens the question "whether the effect of ammonium, glutamate and glutamine are all manifestations of the same regulatory mechanism or are due to different mechanisms" (5).

In this communication we show the occurrence of a second NCR mechanism, usefully designated as "glutamine system", distinct from the previously presented "NH_4^+-gdhA system". They differ by the input signal which for the gdhA system is confirmed as being NH_4^+. The glutamine system in some way involves glutamine synthetase. GDHaseC is regulated by the glutamine system. Allantoinase and urea amido-lyase are under the control of both "NH_4^+-gdhA system" and "glutamine system".

MATERIALS AND METHODS

Strains : All strains derive from wild type Σ1278b. Strains MG1958, MG1951, MG1967 are auxotrophic for glutamine and selected as strain MG935 (gln$^-$), a glutamine auxotroph described previously (3). Strain 12430c is a nonsense mutant gdhA-6. Other strains are presented in ref. 3.

Medium and growth conditions have been described in ref.1. Glucose is the carbon source, nitrogen nutrients are specifically mentioned in each experiment, usually as 1 mg x ml^{-1} as L-isomer ; NH_4^+ as 0.01 M $(NH_4)_2SO_4$. Growth in chemostat was described in ref.2 ; doubling time is 6 hours. When limiting, NH_4^+ is 10^{-3} M and glutamine 0.5×10^{-3}M.

Enzyme assays : enzyme activities are measured following references given in (3), urea amido-lyase as in ref.6. Activities are expressed as µmoles of product formed x hour^{-1} x mg protein^{-1} at 30°C.

RESULTS

Ammonia and glutamine as signals for repression

The level of four enzymes was measured in the wild type strain Σ1278b grown under several conditions of nitrogen nutrition (Table 1, exp. 1 to 7). Glutamine and NH_4^+ cause a similar repression of all enzymes except allantoinase which is less repressed by NH_4^+ than by glutamine. Identification of the primary signal for NCR is difficult due to the interconvertibility of NH_4^+ and glutamine. The case of allantoinase favors glutamine as one signal.

Table 1 : Activities of nitrogen catabolic enzymes in nitrogen catabolic repression mutants

Exp. Nr.	Strains and Genotype	Nitrogen Nutrients in Growth Medium	Arginase	Catabolic (NAD) GDHase	Allan- toinase	Urea amido lyase x 10^{-3}
1	Σ1278b (wild type)	NH_4^+	7	1	0.7	1
2		NH_4^+ + glutamate	8	1	0.35	1
3		NH_4^+ + glutamine	7	1	0.1	1
4		Glutamine	9	0.9	0.1	1
5		Glutamate	50	50	3.5	94
6		Chemostat NH_4^+ limited	35	8	3	115
7		Chemostat glutamine limited	52	16		
8	12430c (gdhA⁻6)	NH_4^+ + glutamate	45	1.3	3.6	
9		NH_4^+ + glutamine	31		1.2	37
10		Glutamine	41	0.5	1	39
11		Chemostat glutamine limited	48	8	4.3	322
12		Chemostat NH_4^+ limited	47	12	3.6	158
13	12597a (gdhCR⁻)	NH_4^+	45	150	8.5	270
14		NH_4^+ + glutamine		120	10	300
15		Chemostat NH_4^+ limited	47	120	4	280
16	0754c (gln⁻11)	Chemostat glutamine limited	42	62		
17		Chemostat glutamine limited, excess (0.02 M) NH_4^+	9	138	2	27
18	MG1958 (gln⁻11,gnrR⁻11)	NH_4^+ + glutamine		50-100	7.6	158
19	MG1950 (gln⁻5,gnrR⁻5)	NH_4^+ + glutamine		1.5	1.2	33
20	MG1949 (gln⁻4)	NH_4^+ + glutamine		0.6	0.1	1
	Tetrad from MG1958x3962c (wild type)					
21	0748a (gln⁻11,gnrR⁻11)	NH_4^+ + glutamine	10	50-100	8.3	95
22	0748b (gln⁺,gnrR⁺)	NH_4^+ + glutamine	10	1.7	0.1	1
23	0748c (gln⁻11,gnrR⁺)	NH_4^+ + glutamine	10	1.7	0.1	1
24	0748d (gln⁺,gnrR⁻11)	NH_4^+ + glutamine	3	0.9	0.43	1
25	0768b (gln⁻11,gnrR⁻11)	NH_4^+ + glutamine		50-100	7	108
26	0794d (gln⁻4,gnrR⁻11)	NH_4^+ + glutamine		50-100	6.6	117
27	0777d (gln⁻5,gnrR⁻11)	NH_4^+ + glutamine		50-100	6.3	122
28	0863b (gdhA⁻6,gln⁻11,gnrR⁻11)	NH_4^+ + glutamine	25	8	7.5	45
29	0833b (gdhA⁻6,gln⁻11,gnrR⁻11)	NH_4^+ + glutamine + glutamate		178		
30	12.759a (gdhCR⁻,gdhA⁻1)	NH_4^+ + glutamine		46	9.9	270
31	0874c (gdhCR⁻,gdhA⁻6,gnrR⁻11,gln⁻11)	NH_4^+ + glutamine		175	8.6	158
32	0842d (gdhCR⁻,gln⁻11,gnrR⁻11)	NH_4^+ + glutamine		200	9.8	144

gln⁻ mutants have been isolated. They lack glutamine synthetase activity ; they can grow with glutamine as sole nitrogen source or with NH_4^+ + glutamine. In the latter case, all four enzymes are repressed (exp.23). A gln⁻ mutant allows to see if NH_4^+ needs conversion into glutamine to exert repression ; if so, NH_4^+ will be inert when added to a culture in a chemostat limited by glutamine. Experiment 17 compared to 16 shows that NH_4^+ represses

arginase completely but it does not repress (NAD) GDHase [*]. In the case of arginase, this result was expected and indeed strengthens the previous proposition that NH_4^+ is the signal of NCR, the (NADP) glutamate dehydrogenase being its receptor acting as a regulatory protein. Insensitivity of (NAD) GDHase to the $gdhA^-$ mutation calls for another mechanism and experiment 17 suggests that not only the receptor molecule is different but also the metabolic signal which does not seem to be NH_4^+ ; repression by NH_4^+ requires conversion into glutamine, so glutamine itself might be a signal.

In a gln^- strain grown in a glutamine-limited chemostat with excess NH_4^+ (exp. 17) urea amido-lyase is less derepressed than in other derepressing conditions (exp. 5 or 6). This suggests that both NH_4^+ and glutamine are signals of repression. Further evidence for this view is the fact that additional derepression occurs in a $gdhA^-$ mutant in chemostat (exp.11) ; this contrasts with the simple behavior of arginase. Allantoinase is affected by the $gdhA^-$ mutation (1) (exp. 8 vs 2 or 9 vs 3) and therefore NH_4^+ is likely to be an effector, but as already mentioned above glutamine is also a possible additional signal. Exp. 9 and 10 show that glutamine partially represses allantoinase synthesized as a result of the $gdhA^-$ mutation in cells grown on NH_4^+ + glutamate (exp.8).

As glutamine (or a derivative) appears to be a primary signal in NCR, mutation of its receptor -distinct from $gdhA^-$ mutation- would be the best indication for an individual glutamine circuit. A first step in the recognition of such a receptor is given below.

Nitrogen catabolite derepression conditioned by the combination of a new ($gnrR^-$) type of mutation and gln^- (glutamine auxotrophy) mutation.

Some 15 new mutants requiring glutamine for growth have been obtained. The glutamine synthetase activity in these mutants is undetectable. Auxotrophy for glutamine is recessive, it segregates as a monogenic character, and it is due to a mutation allelic with the gln^- mutation previously described (3). Some of the mutants have a derepressed level of allantoinase, urea amido-lyase and catabolic (NAD) glutamate dehydrogenase, when grown on NH_4^+ + glutamine. The derepression is variable : high in mutant

[*] Beside the present problem, it must be mentioned that an additional mechanism is involved in (NAD) GDHase regulation. This is obvious when one examines the derepression in a chemostat (exp. 6 and 7), which is much lower than in cells grown on glutamate alone in batch culture (exp. 5). No such discrepancy occurs with other enzymes. A simple process of induction of (NAD) GDHase is not suggested because of the lack of effect of addition of glutamate to ammonium medium (exp. 2 vs 1). The existence of very different levels of derepression from 8 to 180 units, such as in exp. 6, 7, 13, 16 remains an open question.

MG1958 (exp. 18, Table 1), medium in MG1950 (exp.19), it is undetectable in MG1949 (exp.20). Analysis of tetrads resulting from a cross between MG1958 and wild type indicates that derepression is always associated to the gln^- mutation but needs the presence of an additional mutation $gnrR^-$.
An example of a tetrad (tetratype) is given in Table 1 (exp. 21, 22, 23, 24). It can be seen that high derepression is obtained in the double mutant only (0748a). However, a partial derepression of allantoinase is observed in segregant 0748d bearing the $gnrR^-$ mutation only. The $gnrR^-$ type of mutation is also present in the mutant MG1950. The reason for selection of these double mutants is unknown. $gnrR^-$ is used to designate the new mutation to recall its nitrogen catabolite derepressive effect conditioned by the presence of gln^- mutation. Different gln^-,$gnrR^-$ recombinations show that the different levels of derepression observed in the initial mutants MG1958, MG1950 and MG1949 are dictated by the $gnrR^-$ individual mutations. Recombinants 0749d (exp.26) and 0777d (exp. 27) with gln^-4 and gln^-5 mutations issued from mutants MG1949 and MG1950, when combined with $gnrR^-11$ from MG1958 reach the high level of derepression of MG1958, or recombinant 0768b (exp. 18, 25). The gln^-11,$gnrR^-11$ recombinant (0748a) has an arginase level similar to that of the wild type (exp. 21 vs 22 or 1).

We conclude that the nitrogen catabolite repression of some enzymes involves the gnrR and gln genes.

The three enzymes which are derepressed by the gln^--$gnrR^-$ combination are those for which experiment 17 has shown that NH_4^+ is unable to provoke full repression when its transformation into glutamine is prevented. Hence, those enzymes which require glutamine as a signal for NCR are also derepressed by the gln^-,$gnrR^-$ combination. The pool of glutamine in the gln^-11 (strain 0754c) and in the gln^-11,$gnrR^-$ combination (strain 0765c) growing with NH_4^+ + glutamine are identical (72 nomles x mg cells dry weight^{-1}).

In the case of (NAD)GDHase in which repression by NH_4^+ and no derepression by $gdhA^-$ mutation occur, one may conclude to the absence of an NH_4^+-gdhA mechanism. NCR is caused by glutamine with the participation of gln-gnrR genes. For arginase, it is the reverse, there is only indication of an NH_4^+ effect through (NADP) GDHase. For allantoinase and urea amido-lyase, a participation of the NH_4^+-gdhA mechanism is shown by the derepression linked to a $gdhA^-$ mutation as well as by the partial effect of NH_4^+ in exp.17. However, a participation of the glutamine-gln,gnrR mechanism is clearly indicated by the gln^--$gnrR^-$ effect, by the limitation of the NH_4^+ effect, by the reduction of a $gdhA^-$ derepression by glutamine and, in the case of allantoinase, by a stronger repression by glutamine than by NH_4^+ in the wild type.

A recombination of gdhA$^-$, gln$^-$11 and gnrR$^-$11 mutations does not increase derepression (exp.28). The presence of gdhA$^-$ mutation may lower the derepression by shortage in glutamate, indeed addition of glutamate (exp.29) causes high levels of (NAD) GDHase (see footnote *).

The gdhCR$^-$ mutation which is known to cause a high derepression of all four enzymes ((3) and exp. 13, 14) imposes a maximal effect (exp. 30 vs 9 and 14 vs 3). This is expected if the GDHCR molecule issued from gdhCR gene is an obligatory participant acting concomitantly in both NCR mechanisms.

CONCLUSION AND DISCUSSION

The nitrogen catabolite repression (NCR) of the four catabolic enzymes considered in this publication is under the control of two distinct mechanisms acting alone or together. The gdhA mechanism governs the NCR of arginase. It transmits an NH_4^+ signal. The gln-gnrR mechanism governs the NCR of the catabolic glutamate dehydrogenase, it is likely to transmit a glutamine (or derivative distinct from NH_4^+) signal. Allantoinase and urea amido-lyase are under the control of both "NH_4^+-gdhA" and "glutamine-gln, gnrR" mechanism (**)

An element issued from the gene gdhCR is an obligate common regulatory participant for the expression of nitrogen catabolite repression. In enteric bacteria, the glutamine synthetase stimulates the synthesis of enzymes responsible for the degradation of amino acids to glutamate and inhibits the synthesis of glutamate dehydrogenase (8, 9). In S. cerevisiae, the way by which the glutamine synthetase participates to the glutamine circuit presented here is under study.

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(**) The double control of allantoinase and urea amido-lyase is, in part, at the origin of the opinion that the anabolic glutamate dehydrogenase is not involved in the regulation of these enzymes (7), lack of isogenicity among strains used by Bossinger and Cooper increased the confusion (Dubois, Vissers and Wiame, unpublished data).

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