AMMONIUM REPRESSION IN A MUTANT OF *SACCHAROMYCES CARLSBERGENSIS* LACKING NADP DEPENDENT GLUTAMATE DEHYDROGENASE ACTIVITY

K.W. van de POLL
Van 't Hoff Laboratory, Sterrenbos 19, Utrecht, The Netherlands

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1. Introduction

In yeast the synthesis of many enzymes belonging to pathways producing ammonium, such as the NAD dependent glutamate dehydrogenase [1, 2] arginase and ornithine transaminase [3], threonine dehydratase [4] and allantoinase [5, 6], is sensitive to the presence of ammonium in the medium. Moreover in *Neurospora* [7] and *Aspergillus* [8] a number of enzymes in nitrate assimilation and purine breakdown are repressed by ammonium.

In analogy to catabolite repression it can be asked whether there is one mechanism for all these repressing effects, and if so, whether ammonium itself or a metabolite is the effector molecule.

Recently the isolation and characterization were described of mutants of *Saccharomyces* lacking NADP dependent glutamate dehydrogenase activity [9, 10]. It was concluded that this enzyme is responsible for the greater part of the assimilation of ammonium. Consequently it is of interest to study ammonium repression in this *glu* mutant, to verify whether or not an identical repressing effect is observed.

Ammonium repression is studied on the synthesis of allantoinase, which is derepressed under conditions of nitrogen limitation [11]. Although the synthesis of this enzyme can be repressed by many nitrogen sources, derepressed synthesis is most sensitive to the presence of ammonium, asparagine and to a somewhat less extent to the presence of glutamine in the medium [5, 6]. Comparable results were observed for the NAD dependent glutamate dehydrogenase and for glutamine synthetase [12].

2. Materials and methods

In these experiments the following strains derived from *S. carlsbergensis N.C.Y.C. 74* were used: the diploid parent strain with the genotype a/α, lys_2, *LYS*, ade_1, *ADE* and the diploid *glu* mutant, lacking the NADP dependent glutamate dehydrogenase, with the genotype a/α, lys_2, *LYS*, ade_1, *ADE*, *glu*, *glu* [10].

Cells were grown on complete medium, containing 1% yeast extract, 2% bactopeptone and 2% glucose. For the derepression of allantoinase (EC 3.5.2.5) and the induction of the NAD dependent glutamate dehydrogenase (EC 1.4.1.4) cells were harvested in the early logarithmic phase and washed once. Subsequently the cells were resuspended to an absorbance of 0.300 at 590 nm (which is equivalent to 0.5 mg dry wieght of cells per ml medium) and incubated at 30° in a medium containing per ml 2.8 μmoles $MgSO_4$, 7.4 μmoles sodium potassium phosphate buffer (pH 6.2), 0.03% casamino acids and 2% glucose, with or without 10 μmoles glutamate. Allantoinase activity was determined as described before [11]. For the preparation of the cell extracts and all other methods, see [10].

3. Results and discussion

From table 1 it is clear that allantoinase synthesis in cells of the parent strain is repressed by ammonium, asparagine and glutamine. Although the derepressed level of allantoinase is somewhat lower in the *glu* mutant, it is clear that in this mutant allantoinase synthesis is repressed by asparagine and by glutamine, but not by

Table 1
Allantoinase synthesis in the parent strain and in the *glu* mutant.

Additions to the medium	Specific activity of allantoinase (nmoles allantoin split mg^{-1} protein min^{-1})	
	Parent strain	*glu* mutant
None	22	11
2 μmoles/ml $(NH_4)_2SO_4$	2	12
2 μmoles/ml asparagine	1	1
4 μmoles/ml glutamine	3	2
2 μmoles/ml $(NH_4)_2SO_4$ + 10 μmoles/ml glutamate	2	1

Specific acitivity of allantoinase after growth of the cells during 3 hr in a medium as described in the Methods section with additions as indicated below.

Table 2
Synthesis of the NAD dependent glutamate dehydrogenase in the parent strain and in the *glu* mutant.

Additions to the medium	Specific activity of the NAD glutamate dehydrogenase (nmoles NADH split mg^{-1} protein min^{-1})	
	Parent strain	*glu* mutant
10 μmoles/ml glutamate	7.0	4.5
10 μmoles/ml glutamate + 2 μmoles/ml $(NH_4)_2SO_4$	0.5	0.4

Specific activity of the NAD dependent glutamate dehydrogenase after growth of the cells during 3 hr in a medium as described in the Methods section, with additions as indicated below.

ammonium. Recently Dubois et al. [13] found that the synthesis of arginase, allantoinase and of urea amidolyase in a mutant of *S. cerevisiae* lacking NADP dependent glutamate dehydrogenase activity, is insensitive to the presence of ammonium in the medium. However these authors did not test whether the synthesis of one or more of these enzymes can be repressed by asparagine or by glutamine.

These results prove that the presence of ammonium in itself is not enough for ammonium repression, but that it must be metabolized before it can exert a repressing effect. However, the finding that asparagine and glutamine still exert a repressing effect in the *glu* mutant make it unlikely that one of the other reaction constituents of the NADP dependent glutamate dehydrogenase reaction is the effector molecule of ammonium repression on allantoinase synthesis [13].

In the *glu* mutant of *S. carlsbergensis* the synthesis of allantoinase (table 1) and of the NAD dependent glutamate dehydrogenase (table 2) can be repressed by ammonium in combination with higher concentrations of other nitrogen sources, such as glutamate. However this situation complicates the interpretation of ammonium repression on the synthesis of the inducible enzymes, such as the NAD dependent glutamate dehydrogenase: ammonium as the single nitrogen source cannot be tested since the presence of glutamate in the medium is required for the induction of this enzyme [1, 2].

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