YEAST MUTANTS PLEIOTROPICALLY IMPAIRED IN THE REGULATION OF THE TWO GLUTAMATE DEHYDROGENASES

R. Drillien, M. Aigle and F. Lacroute

Laboratoire de Génétique Physiologique
Institut de Biologie Moléculaire et Cellulaire
Rue René Descartes - 67 Strasbourg, France

Received May 17, 1973

Summary : Two different yeast mutant strains are described in which the pattern of regulation of the glutamate dehydrogenases (GDHase) are pleiotropically altered. Both mutants have more than a 20 fold derepreased level of the catabolic GDHase while the level of the anabolic enzyme is three to four fold lower than that of the wild type strain. Data are reported which indicate that the change in the anabolic enzyme is the more significant one.

The utilization by yeast of ammonia or glutamate as nitrogen sources has already been studied (1). Two glutamic dehydrogenases (GDHases) seem to work in opposite directions (2, 3). One is NADP specific and apparently responsible for the synthesis of glutamate (NADP-GDHase ; EC 1.4.1.4.) and the other is NAD specific and apparently responsible for the degradation of glutamate (NAD-GDHase ; EC 1.4.1.2.). Although some aspects of the regulation of these enzymes have also been studied (3, 4), no mutants with an impaired pattern of regulation have yet been described. The enzyme levels of two mutant strains with an abnormal regulation of the GDHases are reported here. Their isolation, genetic and phenotypic characterization have previously been presented (5, 6). One of the mutations (*ure*2) is a semi-dominant mutation, the other (*ure*3) is a cytoplasmically inherited "mutation", which is not linked to the mitochondrial genome.

These two mutants can be phenotypically distinguished from the wild type strain by their capability to utilize ureidosuccinic acid (USA) on an ammonium nitrogen medium. It has been shown that this property is due to the high USA permease activities that these mutants possess on this medium. Moreover, the properties of these mutants suggested that the mutations did not directly affect the regulation of the USA permease. Since the USA permease activity varies with the nitrogen source supplied, it was thought that mutants *ure*2 and *ure*3 could be impaired in their nitrogen metabolism.

MATERIALS AND METHODS :

- Strains : *Saccharomyces cerevisiae*. All strains were derived from the wild type FL100.

Cultures were performed in one liter liquid media at 28°C Overnight and collected in log phase growth.

- The media used were :

. Yeast Nitrogen Base (YNB) without amino-acids = minimal medium with 3,8 10^{-2}M Ammonium Sulfate as sole Nitrogen source.

. Yeast Glutamate Base (YGluB) = minimal medium with 6 10^{-3}M glutamic acid as sole Nitrogen source.

. Yeast Peptone Glucose (YPG) = complete medium (7).

- Enzymes assays : Cell-free extracts were obtained by grinding the cells with glass beads, then centrifuging at 19000 g for 25 min. Enzyme activities were measured by following the decrease in absorbtion at 340 mμ according to Kohllaw, Drägert and Holzer (8), except for the concentration of NADH ($7\ 10^{-4}$M), $NADPH_2$ ($6\ 10^{-4}$M) and α Ketoglutarate (10^{-2}M for NAD-GDHase and $2\ 10^{-3}$M for NADP-GDHase). Protein concentration were measured by the Biuret method. (9).

GDHase levels in the mutant strains. Hierholzer and Holzer (3) showed that ammonium ions repress the synthesis of the NAD-GDHase. In media containing ammonium ions as a nitrogen source the two mutant strains show a very high specific activity for this enzyme (table I-A), about 30 fold the normal activity. This is most likely due to a genetic derepression of the synthesis of the enzyme. With regard to the NADP-GDHase, its activity is only one third that of the wild type strain. Thus these two mutations affect pleiotropically the activities of the two GDHases. If the strains are cultured with glutamate as the nitrogen source (table I-B), again different specific activities are found for the mutant and wild type strains although these differences are slighter. The normal derepression of the NAD-GDHase of the wild type strain reaches a value three to four times less the level of the mutant strains. There is also a 50 percent drop in the NADP-GDHase level of the wild type strain which results in a decrease in the difference with the mutants.

Thomulka and Moat (4) found that the level of the anabolic enzyme is lowered on a complete medium. With our wild type strain the activity of the NADP-GDHase is about 20 fold smaller on a complete medium than on the minimal medium while the activity of the mutants is not as strongly repressed (table I-C). It will be possible to interpret this result only when the substance or substances involved in the repression will be characterized (it is likely that the NADP-GDHase is subject to a cumulative repression).
The complete medium contained ammonia and as should be expected the NAD-GDHase activity is repressed in the wild type strain, thus reaching the same level as

Table I. : Specific activities of the strains (μmoles $NADH_2$ or $NADPH_2$/min./mg protein).

A. Cultured in YNB.

Genotype	NAD-GDHase	Rel•	NADP-GDHase	Rel•
(+)	53	1	685	1
(*ure*2)	1609	30	201	0.29
(*ure*3)	1782	34	190	0.27

B. Cultures in YGluB.

Genotype	NAD-GDHase	Rel•	NADP-GDHase	Rel•
(+)	830	1	322	1
(*ure*2)	3048	3.6	206	0.6
(*ure*3)	2870	3.4	225	0.7

C. Cultured in YPG.

Genotype	NAD-GDHase	Rel•	NADP-GDHase	Rel•
(+)	60	1	31	1
(*ure*2)	3354	56	79	2.5
(*ure*3)	2935	49	76	2.4

These results were obtained in the same experiment.

• Values calculated with the wild type as unity.

on the minimal medium. Moreover, in the mutant strains, the activity of the NAD-GDHase reaches 50 fold that of the wild type extract, which has the same level as on the glutamate medium.

Table II. : Specific activity in the heterozygous strains (culture in YNB).

Genotypes	NAD-GDHase	Rel	NADP-GDHase	Rel
Exp. 1				
(+/+)	35	1	417	1
(*ure2/ure2*)	639	18	104	0.2
(*ure2*/+)	35	1	319	0.8
Exp. 2				
(+/+)	57	1	491	1
(*ure3*/+)	997	17	152	0.3
(*ure3/ure3*)	1106	19	163	0.3

Table III. : Specific activity in the recombinant strains with the *ngl3* mutation. (culture in YNB)

Genotypes	NAD-GDHase	Rel	NADP-GDHase	Rel
(+)	47	1	547	1
(*ure2*)	1419	30	192	0.35
(*ure3*)	2069	44	248	0.45
(*ngl3*)	21	0.44	664	1.2
(*ure2-ngl3*)	18	0.39	244	0.45
(*ure3-ngl3*)	16	0.34	200	0.36

Results concerning the heterozygotes. The *ure2* mutation exhibits a semi-dominant phenotype (5) and the *ure3* "mutation" a complete "dominance" (6).
The specific activities of the two enzymes were measured in the heterozygous strains (see table II).
Here, the mutant strains show in agreement with their phenotypes, two different behaviours.

The (*ure*3/+) "heterozygote" has the same activity as the (*ure*3/*ure*3) homozygote. On the other hand, the (*ure*2/+) heterozygote has a surprising pattern of activity : the NAD-GDH activity decreases to reach the wild type level, whereas the activity of the NADP-GDH is significantly lower than that of the wild type. This level is nevertheless higher than the level calculated for a simple mechanism of genic balance.
The semi-dominant phenotype of the *ure*2 mutation can thus be found in the regulation of the two GDHases, the regulatory change for one of the enzymes is recessive while that for the other is not.

Relation with a mutation involved in NAD-GDHase regulation. Another mutation, named *ngl*3, affecting the regulation of the NAD-GDHase has been obtained (M. Grenson and al. - to be published). A mutant strain *ngl*3 exhibits poor growth on a glutamate nitrogen medium ; this phenotype can be correlated with the lack of derepression of the NAD-GDHase on the above medium. Strains carrying both the *ngl*3 mutation and the *ure*2 or *ure*3 mutation have been constructed (*ure*2 and *ngl*3 are not genetically linked), and the GDHase activities determined (table III). Here also, the derepression of the NAD-GDHase on an ammonium medium disappears as in heterozygotes of *ure*2. With the *ure*3 "mutation" this effect is identically suppressed. However, the NADP-GDHase levels in the recombinant strains are not altered by the *ngl*3 mutation.

DISCUSSION : The results obtained do not allow us to propose a mechanism of action of the *ure*2 and *ure*3 mutations and consequently a model of the genetic regulation of the enzymes involved, but some characteristics can be defined. Two different kinds of mutations affect in a similar manner the regulation of the two GDHases. This suggests the existence of a common element, either metabolic or genetic, in the control of the levels of these two enzymes. It should also be beared in mind that the mutations described could modify quite indirectly the GDHase levels. Moreover, the fact that the NADP-GDHase level is unaltered in the heterozygote *ure*2/+ and in the recombinant with *ngl*3, while the NAD-GDHase level changes, indicates that the primary effects of the mutations could be on the NADP-GDH gene. However it should be noted that the *ure*2 mutation is not linked to the structural gene for the NADP-GDHase (unpublished results). Concerning the *ure*3 "mutation", its extrachromosomal inheritance and the fact that its phenotype is strictly identical to the chromosomal mutation *ure*2 allow one to imagine that the same regulatory mechanism has been impaired at a different biochemical level in the two cases ; if these different levels could be identified, this could give an important clue about the special features of eucaryotic cell regulation.

No clear cut relationship has been found between the high USA permease activities in the *ure*mutants and the GDHase levels. Measurements of the amino-acid pools (unpublished results) indicate that glutamate can not be the only molecule involved in this regulation. It is however obvious that the problem concerning the regulation of this permease must be included in the general subject of nitrogen catabolic repression (10, 11).

Mutations in the structural gene for the NADP-GDHase have already been found (12) (Grenson and al., to be published) and the properties of the NADP-GDHase have been studied with a purified enzyme (13,14). Similarly the isolation of a mutation in the structural gene for the NAD-GDHase and the purification of this enzyme would be other important steps in our understanding of the yeast nitrogen metabolism.

ACKNOWLEDGEMENTS : This work was supported in part by contract n° 71.1047.2 from Institut National de la Santé et de la Recherche Médicale and by a grant from the Fondation pour la Recherche Médicale Française.

REFERENCES :

1) Witt I. und H. Holzer (1964) - Bioch. Zeit. 339 ; 255-265
2) Holzer H. und S. Schneider (1957) - Bioch. Zeit. 329 ; 361-369
3) Hierholzer G. und H. Holzer (1963) - Bioch. Zeit. 339 ; 175-185
4) Thomulka and Moat (1972) - J. Bacteriol. 169 ; 25
5) Drillien R. and F. Lacroute (1972) - J. Bacteriol. 109 ; 1
6) Lacroute F. (1971) - J. Bacteriol. 106 ; 2
7) Meuris P., F. Lacroute and P.P. Slonimski (1967) - Genetics 56 ; 149-161
8) Kohllaw G., W. Drägert und H. Holzer (1965) - Bioch. Zeit. 341 ; 224-238
9) Stickland (1951) - J. Gen. Microbiol. 5 ; 698
10) Wiame J.M. (1971) - Current topics in cellular regulations - 4 (Academic Press)
11) Dubois and M. Grenson (1973) - Biochem. Biophys. Res. Commun. 50 n° 4 ; 967-972
12) Grenson M. and C. Hou (1972) - Biochem. Biophys. Res. Commun. 48 n°4 ; 749-756
13) Fourcade A. (1968) - Bull. Soc. Chim. Biol. 50 ; 1671
14) Fourcade A. (1972) - Biochimie 54 ; 1381-1389