POSSIBLE FAILURE OF NADP-GLUTAMATE DEHYDROGENASE TO PARTICIPATE DIRECTLY IN NITROGEN REPRESSION OF THE ALLANTOIN DEGRADATIVE ENZYMES IN *SACCHAROMYCES CEREVISIAE*

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SUMMARY: The extent of repression exerted by various nitrogen-containing compounds was measured in wild type and NADP-GDH defective strains of *Saccharomyces*. We found that in strains carrying the $gdhA_6^-$ mutation, repression was relieved only for compounds whose metabolism involved the intermediate generation of ammonia. This raises the possibility that relief of repression observed in these strains is the result of their failure to effectively metabolize ammonia rather than an inability of the NADP-GDH protein to function as a regulator itself.

Wiame and his associates have reported (1-6) that NADP-specific glutamate dehydrogenase (NADP-GDH), when complexed to ammonia and α-ketoglutarate, directly participates as the control element for the nitrogen repression of arginase in *Saccharomyces cerevisiae*, thus playing a role analogous to that clearly documented (7,8) for glutamine synthetase in *Klebsiella aerogenes*. UALase (urea amidolyase or the urea carboxylase-allophanate hydrolase multienzyme complex) was also reported to be regulated in this manner by NADP-GDH. Our studies concerning gene expression of the allantoin degradative system prompted us to verify Wiame's result. Unfortunately, the data we obtained do not apparently support the earlier conclusions.

METHODS

Two strains have been used in this work. M-25 is our standard wild type diploid strain which has been described in detail (9-12). A second diploid strain carrying the $gdhA_6^-$ nonsense (ochre) mutation (4) in homozygous condition was constructed by crossing a haploid strain (12430C) containing the $gdhA_6^-$ lesion to one of our wild type haploid strains (M-25-12B). A spore with the $gdhA_6^-$ mutation was selected at random and crossed back a second time to our wild type haploid strain (S-185). From this cross a and α spores were selected carrying appropriate overlapping auxotrophies and the $gdhA^-$ mutation. These were then mated to yield the homozygous $gdhA_6^-$ diploid strain used in our studies. The lack of NADP-GDH activity in this strain was verified biochemically by assaying enzyme activity before it was used experimentally.

Wickerham's medium (9,13) was used throughout this work. The nitrogen source used is that indicated in the Table at a final concentration of 0.1%, and glucose at 0.6% final concentration was used in all cases as sole carbon source. Differential rates of allophanate hydrolase production were determined as reported earlier (11) using oxaluric acid (OXLU) as a non-metabolizable inducer of the system (10).

RESULTS AND DISCUSSION

Table I summarizes the differential rates of allophanate hydrolase production observed in strains growing on various nitrogen sources. In strains carrying the $gdhA_6^-$ mutation repression was alleviated only when metabolism of the nitrogen source involved the NADP-GDH enzyme activity. Therefore, as expected, there is less repression in the case of ammonia, glutamine, and asparagine, and no relief is observed in the remaining cases. If liberation from repression is derived only from an inability to metabolize ammonia, it would be expected that the rates of allophanate hydrolase production would be about the same in NADP-GDH defective cells growing on either glutamate or glutamine, assuming, of course, that rates of entry for both compounds are similar and that the non-metabolized ammonia pool has no serious deleterious effects. As shown in Table I, this was observed for glutamine and glutamate and also for asparagine and aspartate although with less consistency in the latter case.

These data argue that relief of nitrogen repression observed in NADP-GDH defective strains is most likely the result of their inability to effectively metabolize ammonia provided exogenously or produced during degradation of an amino acid rather than failure of the defective enzyme itself to function as a regulator. In addition, some repression by glutamate and aspartate is still evident in the mutant strain which contains a nonsense mutation in NADP-GDH and, therefore, presumably lacks the NADP-GDH protein. This again argues an indirect rather than direct role for NADP-GDH in nitrogen repression in agreement with the case presented for another fungi, *Aspergillus nidulans* (14). We are presently carrying out a detailed study to reconcile the two opposing sets of data. Such a study is essential because these facts require either

TABLE I

Repression of Allophanate Hydrolase Production in Wild Type and NADP-GDH Defective Strains of *Saccharomyces cerevisiae*

Nitrogen Source	Increase in Enzyme Activity* Per 10 Klett Units of Growth	
	Wild Type	NADP-GDH Minus
Proline	2.20	1.95
Ammonia	1.46	1.62
Glutamine	0.25	0.53
Glutamate	1.13	0.47
Asparagine	0.01	0.73
Aspartate	0.73	0.47

*Activities are expressed as nmoles of product produced per minute per milliliter of culture.

that separate mechanisms be proposed for the repressive effects of ammonia and amino acids upon synthesis of allophanate hydrolase and arginase or that the proposed direct participation of NADP-GDH in nitrogen repression be re-evaluated.

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