INDUCTION OF THE ALLANTOIN DEGRADATIVE ENZYMES BY ALLOPHANIC ACID, THE LAST INTERMEDIATE OF THE PATHWAY

Terrance G. Cooper and Robert Lawther
Department of Biochemistry, F.A.S.
University of Pittsburgh
Pittsburgh, Pennsylvania 15261

Received March 16, 1973

SUMMARY

Saccharomyces cerevisiae can utilize allantoin as a sole nitrogen source by degrading it in five steps to ammonia, "CO_2", and glyoxylate. We have previously shown that allophanic acid is the inducer of the urea carboxylase: allophanate hydrolase multienzyme complex. Since these enzymes catalyse the last two steps of allantoin degradation, experiments were performed to determine if allophanate was also the inducer of any other enzymes in the pathway. Our data demonstrate that allophanate induces synthesis of at least five of the seven purine degradative enzymes.

Saccharomyces cerevisiae requires large quantities of biotin when using allantoin, allantoic acid, or urea as sole nitrogen source (1). An explanation for this observation was found in Levenberg's demonstration that a urea degradative activity in *Candida utilis* was sensitive to avidin (2). Our experiments and those of others (3-7) provided evidence that this degradative function was performed by a multienzyme complex, composed of a biotin containing urea carboxylase and an avidin insensitive allophanate hydrolase (reactions 4 and 5 in Fig. 1).

Since these enzymes are found only in cultures grown in the presence of urea, arginine or a purine, we sought the identity of their inducer. We found (8) that: (1) strains lacking allophanate hydrolase possessed a high constitutive level of urea carboxylase whether or not the culture was grown in the presence of urea, whereas strains lacking urea carboxylase produced a basal level of allophanate hydrolase which did not increase upon the addition of urea; (2) formamide and hydantoic acid induced these activities in wild type strains, but would not serve as nitrogen sources; and (3) formamide, a urea analogue, was not capable of inducing the hydrolase in a carboxylase minus strain, whereas hydantoate, an allophanate analogue, was able to induce

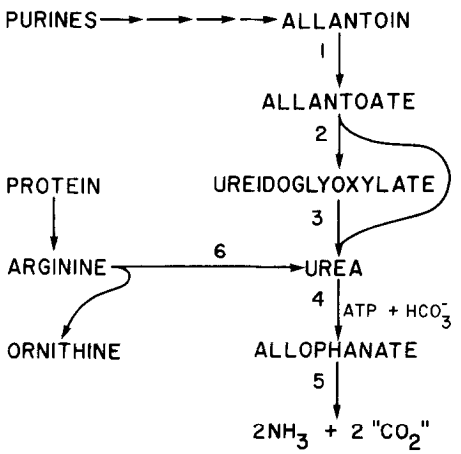

Figure 1. Reactions involved in the degradation of arginine and allantoin in *Saccharomyces cerevisiae*.

the hydrolase in such a strain. These data suggested that the production of urea carboxylase and allophanate hydrolase is contingent upon the presence of allophanic acid, a product of the urea carboxylase reaction.

Although urea carboxylase and allophanate hydrolase may be thought of as anabolic activities functioning in the assimilation of urea nitrogen, they also may be considered as the last two steps in the degradation of arginine and purines (Fig. 1). In view of these considerations, it was pertinent to identify the inducer of the other enzymes participating in the degradation of allantoin. The inducer of arginase (reaction 6 of Fig. 1) has been shown to be arginine (10). The data presented here support the contention that allophanate, the last intermediate of the degradative pathway is the inducer of at least five of the seven enzymes unique to purine degradation.

METHODS

Data substantiating the biochemical phenotypes of mutant strains used in this work appears elsewhere (4,7,9). All of the strains were prototrophic diploids, homozygous for the significant alleles. The physiological experiments were conducted using a protocol similar to that reported earlier (3,11), except the concentration of $(NH_4)_2SO_4$ in the media was 0.1%. The final con-

TABLE I

DIFFERENTIAL RATE OF ALLANTOINASE SYNTHESIS IN WILD TYPE AND MUTANT STRAINS OF *Saccharomyces cerevisiae*.

Inducer Added to Growth Medium	M-25 Wild Type	M-62 Urea Carboxylase Defective	M-64 Allophanate Hydrolase Defective	M-130 Allantoicase & Arginase Defective	M-58 Arginase Defective
	Increase in Enzyme Activity[a] per 10 Klett Units of Growth				
None	75	66	375	50	42
Allantoin	575	66	---	88	--
Allantoate	575[b]	58	---	114	--
Urea	575[b]	66	---	475	225
Formamide	575[b]	62	---	---	--
Arginine	238[b]	--	---	---	13

[a]Activities are expressed in nmoles glyoxylate produced per minute per ml of culture. The values are the slopes of induction curves obtained with log phase cultures.

[b]Data from two separate experiments were used and normalized to the urea value.

centration of the various inducers was 10^{-2}M. Allantoinase was assayed by the methods of Van de Poll, *et al* (12).

RESULTS

Table 1 summarizes the results of experiments that test the ability of various compounds to serve as inducers of allantoinase in strains defective in one or more of the activities depicted in Fig. 1. It is clear that allantoin will serve as an inducer only in those instances where it can be converted to allophanic acid. Essentially the same behavior was observed for allantoic acid and urea. The fact that none of these compounds are effective in a mutant lacking urea carboxylase suggests that this enzyme or its product allophanate, is vital to induction of allantoinase.

This hypothesis was tested by monitoring allantoinase activity in an allophanate hydrolase defective strain. *Saccharomyces cerevisiae* is known to possess a very large arginine pool. Degradation of this amino acid in a hydrolase minus strain would be expected to increase the intracellular concentration of allophanate. Therefore, if allophanate is the inducer of allantoinase, a hydrolase defective mutant should contain high levels of allantoinase in the absence of added urea. As shown in Table 1, this result was observed. Formamide, an analogue of urea, has been shown to bring about induction of the urea degradative multienzyme complex in wild type strains, but not in strains lacking urea carboxylase. This analogue was shown (4) to be carboxylated and was suggested to function as an inducer of the complex only after being converted to carboxyformamide, an allophanate analogue. Formamide was tested for its ability to induce allantoinase and was demonstrated (Table 1) to bring about its synthesis. Experiments similar to those reported here have been performed (9) with allantoicase and ureidoglycolate hydrolase. These two enzymes appear to behave in a manner similar to that of allantoinase. It is significant that the fold induction of allantoinase greatly increases when the intracellular sources of urea (arginase and purines) are blocked by appropriate mutations. These mutations presumably decrease the level of allophanate which

results in a lower differential rate of allantoinase synthesis in uninduced cells. It must also be pointed out that the slight induction observed with allantoic acid is caused by urea which has been shown (Sumrada and Cooper, unpublished) to contaminate all commercial preparations of this compound.

DISCUSSION

Our results show that synthesis of at least five of the seven enzymes participating in purine degradation is contingent upon the presence of allophanate. Palleroni and Stanier (13) demonstrated that the inducer of the first two enzymes of tryptophan degradation is kynurenine, a product of the second enzyme in the pathway and Schlesinger, *et al* (14) showed that the enzymes of the histidine degradative pathway are induced by urocanic acid, product of the first enzyme of the pathway. However, this is the first example, in either procaryotic or eucaryotic systems, of an inducer being the last intermediate in a degradative pathway. The physiological value of this situation resides in the buffering effect upon induction that is gained by having an inducer be the last intermediate in a multi-step pathway. In the presence of excess nitrogen it is unnecessary for an organism to degrade its intracellular pools of purine related metabolites. In this situation the remoteness of the inducer from the initial reactions would assure that its concentration remained at a minimum value. On the other hand, during periods of nitrogen limitation *Saccharomyces* has been shown to mobilize its large pools of arginine (10). This results in a dramatic increase in the intracellular levels of urea and allophanate. It is presumably during these periods of nitrogen limitation that it would be beneficial to metabolize any excess nitrogenous compounds available. The central position of allophanate at the terminal junction of arginine and allantoin catabolism allows it to function as a major, unique control element of nitrogen metabolism in *Saccharomyces cerevisiae*.

ACKNOWLEDGMENT

This work was supported by NIH grant GM-19386, Health Research and Services Foundation Grant 0-50 and a Brown-Hazen grant-in-aid to T. G. Cooper. R. Lawther was supported by a Andrew Mellon Predoctoral Fellowship award.

REFERENCES

1. DiCarlo, F.J., Schultz, A.S., and Kent, A.M., *Arch. Biochem. Biophys.*, *44*, 468-474 (1953).
2. Roon, R.J. and Levenberg, B., *J. Biol. Chem.*, *243*, 5213-5215 (1968).
3. Whitney, P.A. and Cooper, T.G., *J. Biol. Chem.*, *247*, 1349-1353 (1972).
4. Whitney, P.A. and Cooper, T.G., *J. Biol. Chem.*, *248*, 325-330 (1973).
5. Thompson, J.F. and Muenster, A.E., *Biochem. Biophys. Res. Commun.*, *43*, 1049-1055 (1971).
6. Roon, R.J., Hampshire, J., and Levenberg, B., *J. Biol. Chem.*, *247*, 7539-7545 (1972).
7. Whitney, P.A. and Cooper, T.G., *Biochem. Biophys. Res. Commun.*, *49*, 45-51 (1972).
8. Whitney, P.A., Cooper, T.G., and Magasanik, B., *J. Biol. Chem.*, Submitted for publication (1973).
9. Lawther, R. and Cooper, T.G., In preparation (1973).
10. Whitney, P.A. and Magasanik, B., *J. Biol. Chem.*, Submitted for publication (1973).
11. Whitney, P.A. and Cooper, T.G., *Biochem. Biophys. Res. Commun.*, *40*, 814-819 (1970).
12. Van de Poll, K.W., Verwey, A.A.G., Koningsberger, V.V., *Proc. Kon. Ned. Akad. wet.*, *B71*, 344-358 (1968).
13. Palleroni, N.J. and Stanier, R.Y., *J. Gen. Microbiol.*, *35*, 319-334 (1964).
14. Schlesinger, S., Scotto, P., and Magasanik, B., *J. Biol. Chem.*, *240*, 4331-4337 (1965).