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Control of Lysine Biosynthesis in Yeast[1]

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The inhibition of the pathway of biosynthesis of lysine in yeast has been studied *in vivo*. The site sensitive to inhibition at low concentrations of lysine is after α-aminoadipate on the pathway. A test was made for a branch point in the pathway, but no evidence was obtained to demonstrate a branch point.

In the accompanying paper (1) we have shown that there are two homocitrate synthases in yeast and that both enzymes are sensitive to feedback inhibition at relatively high concentrations of lysine. In contrast to these results, we have shown in a brief report (2) that *in vivo* the pathway as a whole is sensitive to inhibition by low concentrations of lysine. The *in vivo* studies are extended in the present report. The pathway of lysine biosynthesis is sensitive to inhibition by lysine at more than one site. The first enzyme, homocitrate synthase, is sensitive to feedback inhibition at high lysine concentrations. A later site, after α-aminoadipate, is sensitive to lysine inhibition at much lower concentrations.

These results, and a number of other observations are consistent with there being a branch point in the pathway. We have tested this hypothesis by constructing a double mutant, but have found no branch point in the pathway.

MATERIALS AND METHODS

All materials and methods not described here are described in the accompanying paper (1). The lysine auxotrophs and wild type of *Saccharomyces* were from the Lindegren collection: Strain S288C (ly_1) mating type α, 19B (ly_4) mating type a, and WL-1 (wild type). Our initial sample of saccharopine was a gift of Dr. M. Ogur, Department of Microbiology, Southern Illinois University, Carbondale. Saccharopine was also isolated from commercial baker's yeast by the method of Darling and Larsen (3). [6-^{14}C]α-Aminoadipate was purchased from Dr. Leon Miller, Rochester, NY. The complete synthetic minimal medium used was the minimal medium described by Maragoudakis and Strassman (4). For the medium used to test for a vitamin requirement the minimal vitamin mixture of Burkholder *et al.* (5) contained biotin (0.2 mg), β-alanine (100 mg), inositol (100 mg), and thiamine (40 mg) in 100 ml. An aliquot of 0.1 ml of this mixture was added to each liter of medium instead of the usual vitamin mixture. Sporulation and complete media were as described by Fink (6). Homoaconitase was assayed by the method of Strassman and Ceci (7). Saccharopine dehydrogenase was assayed in the forward and reverse directions by the method of Saunders and Broquist (8).

To measure lysine inhibition *in vivo*, the incorporation of radioactivity into lysine from [1-^{14}C]acetate or [6-^{14}C]α-aminoadipate was determined in growing cultures of yeast. Wild-type (WL-1) yeast was grown in 50 ml of synthetic minimal medium in 250-ml Erlenmeyer flasks at 30°C. When the cells were growing at an exponential rate, radioactive precursor and various concentrations of lysine were added to each flask. After 15 min of incubation, growth was terminated by the addition of hydrochloric acid to a final concentration of 6 N, and 10 mg of L-lysine was added as a carrier. The total of free plus protein-bound lysine of the medium plus the cells was isolated and the radioactivity incorporated was determined by the method of Tucci (2).

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The double mutant ly_1ly_4 was prepared as follows: An inoculum of approximately 10^6 of each of the opposite mating types of ly_1 (α), and ly_4 (a) were mixed and incubated in 10 ml of complete medium for 16 hr. The cells were centrifuged, washed, and transferred to sporulation medium for 3 days. Spores were observed under the microscope. The culture was heated at 55°C for 10 min to kill haploid cells. Aliquots were transferred to complete liquid medium to which aminoadipate (60 mg per liter) had been added. After growth for 1 day on this medium, the cells were plated onto complete medium supplemented with aminoadipate at a density of 50–100 colonies per plate. The plates were replica plated onto minimal medium, and minimal plus lysine (30 mg per liter) and aminoadipate (60 mg per liter). Colonies that did not grow on the minimal medium but grew on the other medium were scored, picked from the plates, and grown on 500 ml of complete liquid medium supplemented with aminoadipate.

The cells were harvested and washed, and then broken in a centrifuge shaker, as described previously (1). Extracts were tested for homoaconitase and saccharopine dehydrogenase activity. The cells corresponding to the extract having neither activity was identified as ly_1ly_4.

An inoculum of 5×10^5 cells of ly_1ly_4 was transferred to 50 ml of various synthetic media in 250-ml Erlenmeyer flasks equipped with a side arm to monitor growth by turbidity measurements at 550 nm. For the test for a vitamin requirement the cells were grown to mid-log phase, and then a 0.1-ml aliquot was transferred to a fresh culture. This process was repeated through 10 transfers.

RESULTS AND DISCUSSION

Addition of lysine reduced the incorporation of radioactivity into lysine from 10 μCi of [1-^{14}C]acetate in wild-type yeast. There was 47,600 cpm incorporated in 15 min into lysine in the flask with no lysine added. As is shown in Fig. 1, the incorporation of radioactivity into lysine was inhibited 80% at high concentrations of lysine, and half-maximal inhibition (40%) was obtained at a concentration of between 1 and 2×10^{-5} M lysine in the medium. In a similar experiment the cells were incubated in the presence of lysine and radioactive acetate for 60 min instead of 15 min. There were 186,000 cpm incorporated into lysine in the control flask. A maximal inhibition of 79% was obtained at 1×10^{-3} M and higher concentrations, and half-maximal inhibition (40%) was obtained at approximately 5×10^{-5} M lysine. The similarity of the results at 60 min to those at 15 min indicated that the reduction of the inhibition

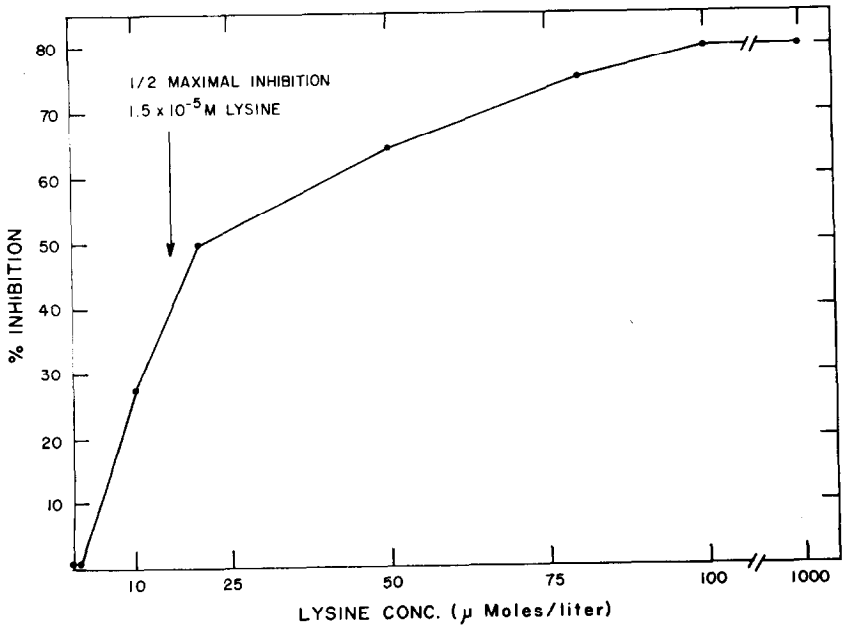

FIG. 1. The effect of lysine on the rate of incorporation of radioactivity into lysine from [1-^{14}C]acetate in yeast in 15 min.

at low concentrations of lysine probably was not due to exhaustion of lysine from the medium in the experiment with a 15-min incubation. We have already established (1) that homocitrate synthase is not the site of this inhibition that is sensitive to low concentrations of lysine.

To further locate the site of inhibition of the pathway by lysine, use was made of radioactive α-aminoadipate to determine if the latter half of the pathway was sensitive to lysine inhibition. Wild-type cells were grown in minimal medium and incubated with [6-^{14}C]α-aminoadipate (10 μCi/50 ml) in the presence of varying concentrations of lysine for 15 min, and incorporation of radioactivity into lysine determined as described for the previous experiments.

TABLE I

THE EFFECT OF LYSINE ON THE INCORPORATION OF RADIOACTIVITY INTO LYSINE FROM [6-^{14}C]α-AMINOADIPATE IN WILD-TYPE YEAST GROWN ON MINIMAL MEDIUM

Lysine added to medium (M)	Incorporation into lysine (cpm)	% inhibition
0	73,000	—
2×10^{-5}	62,500	15
4×10^{-5}	52,000	28
6×10^{-5}	50,600	31
8×10^{-5}	35,000	52
2×10^{-4}	21,900	70

As shown in Table I, lysine inhibited the incorporation of radioactivity from radioactive aminoadipate to lysine. Moreover, the inhibition obtained was only slightly less for any given lysine concentration than had been obtained when starting with radioactive acetate. These results are consistent with the view that the most significant site of inhibition of the pathway is after aminoadipate. Certainly the feedback inhibition exerted on homocitrate synthase (1) must play a minor role in the control of the pathway *in vivo* because of the high concentrations of lysine required to demonstrate it, and the low concentrations of lysine that

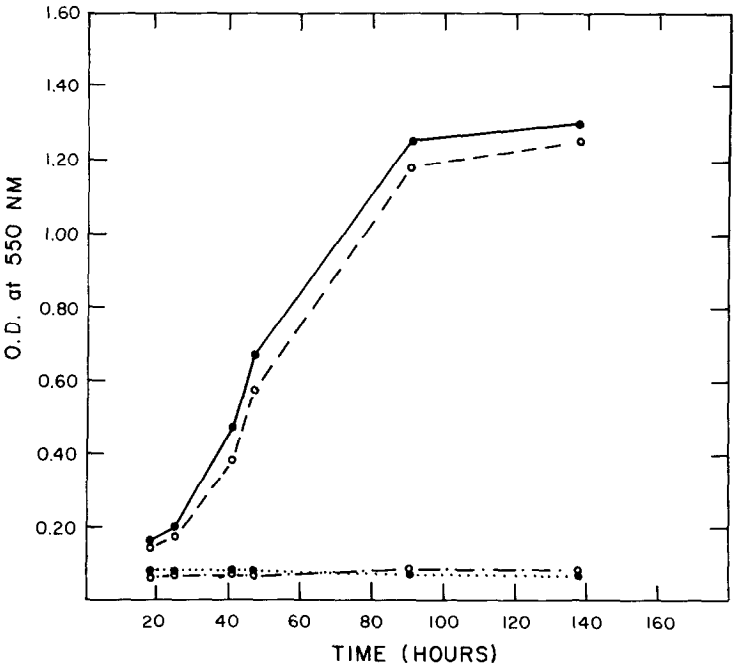

FIG. 2. Growth of double mutant ly_1ly_4 on various media. ●.....●, minimal medium. O...O, minimal medium supplemented with α-aminoadipate (60 mg/liter). ●——●, minimal medium supplemented with lysine (30 mg/liter). O----O, minimal medium supplemented with lysine and α-aminoadipate. Turbidity measurements taken at 550 nm with an 18-mm light path.

can effect an inhibition of the latter half of the pathway.

A sensitive site in the middle of a pathway frequently occurs when there is a branch to a pathway. The presence of two homocitrate synthases, one of which is repressible, the other less so, is also consistent with there being a branch in the pathway. In other organisms that utilize this pathway there is evidence that the pathway may be branched. Snyder and Broquist (9) have shown that in the biosynthesis of slaframine in *Rhizoctonia leguminicola* intermediates of this pathway, such as aminoadipate, are precursors. In *Penicillium chrysogenum* the synthesis of lysine and penicillin are linked since aminoadipate is involved in penicillin biosynthesis, the tripeptide aminoadipylcystinylvaline being a penicillin precursor (10). In addition, excess lysine in the growth medium causes a reduction in the production of penicillin. Therefore, a branch in the pathway exists in *Penicillium*.

We devised a test for a branch in the pathway in yeast. A mutant blocked early in the pathway might get to the branch point by a reversal of the reactions from lysine to the intermediate at the branch point. A mutant blocked late in the pathway would be able to synthesize the intermediate at the branch point. Therefore, both mutants might require only lysine for growth. However, a double mutant, blocked both early and late in the pathway, should not be able to grow on lysine alone if the other final product were a compound essential for growth. We prepared the double mutant ly_1ly_4, verified that it lacked both homoaconitase and saccharopine dehydrogenase activity, indicating that it was blocked at the second and last steps in the pathway. In Fig. 2 we see the results of this double mutant being grown on various media. The growth on lysine alone was as good as on lysine plus aminoadipate, but on minimal medium and on minimal medium plus aminoadipate it did not grow. If there were a branch to the pathway the double mutant should have grown only on the medium containing both lysine and aminoadipate. We also grew this double mutant on a minimal medium containing the minimal vitamin requirements and lysine through 10 transfers and many more generations to eliminate the possibility that a vitamin requirement had been missed, but none was apparent, since the cells grew well on this medium. No evidence was obtained for a branch in the pathway, but of course a branch could exist if the other end product were not essential for growth, or if there were an alternative pathway to its synthesis, or if there were a branch point after the first step in the pathway.

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