α-AMINOADIPIC ACID AS A PRECURSOR TO LYSINE IN NEUROSPORA*

By EMANUEL WINDSOR

(*From the Kerckhoff Laboratories of Biology, California Institute of Technology, Pasadena, California*)

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In a recent investigation reported by Mitchell and Houlahan (1) it was shown that a mutant strain of *Neurospora crassa*, strain 33933, was able to utilize α-aminoadipic acid in place of lysine. Although these authors interpreted their results as showing that α-aminoadipic acid was a precursor in the biosynthesis of lysine by *Neurospora*, other pathways are conceivable.

During the isolation of α-aminoadipic acid from a natural protein radioactive α-aminoadipic acid was added as a marker for chromatographic analysis (2). In order to identify the isolated amino acid an aliquot was provided as a growth substance for the *Neurospora* mutant 33933. Thus the opportunity was available to follow the radioactivity and determine whether or not α-aminoadipic acid was a precursor to lysine.

EXPERIMENTAL

The ε-C^{14}-α-aminoadipic acid[1] diluted with isolated α-aminoadipic acid had an activity of 265 c.p.m. per mg. (corrected) or 42.7 c.p.m. per μM. Varying quantities, totaling 8.6 mg., were added to 20 ml. each of minimal medium in flasks to obtain a growth curve with *Neurospora* mutant 33933 (Fig. 1). The pooled mycelia, weighmg 87.5 mg., were ground to a powder and hydrolyzed with 40 ml. of 20 per cent HCl on an oil bath for 20 hours, and the excess HCl was repeatedly evaporated *in vacuo*. The syrupy hydrolysate was made up to 0.6 ml., and an aliquot of 0.2 ml. was put along the bottom of a sheet of Whatman No. 1 filter paper, according to the ascending technique of Williams and Kirby (3). Spots of α-aminoadipic acid and lysine were also put on both sides of the hydrolysate, and the chromatogram developed for 16 hours with phenol saturated with water. The paper was dried at 100° and that part containing the known lysine and α-aminoadipic acid, as well as the edge of the part containing the hydrolysate, was sprayed with 0.1 per cent ninhydrin solution in butanol saturated with water. The color was developed at 100° for 5 minutes.

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[1] Generously contributed by Dr. H. Borsook and Dr. P. H. Lowy.

That part of the paper containing lysine from the hydrolysate was cut out, the lysine eluted with several small portions of water, and the specific activity of the lysine determined. Microbiological assay[2] of the lysine with *Leuconostoc mesenteroides*, which has been shown to be unaffected by the presence of α-aminoadipic acid (4, 5), and use of a Geiger-Müller counter gave a specific activity of 306 c.p.m. per mg., or 44.7 c.p.m. per μM. Within experimental error the activity was identical with that of the α-aminoadipic acid used, 42.7 c.p.m. per μM.

That region of the paper chromatogram containing α-aminoadipic acid was cut out, the amino acid eluted with small portions of water, and the water evaporated. The residue had only 9.5 c.p.m. (corrected), corresponding to 0.11 mg. of α-aminoadipic acid or less than 1.3 per cent of that provided.

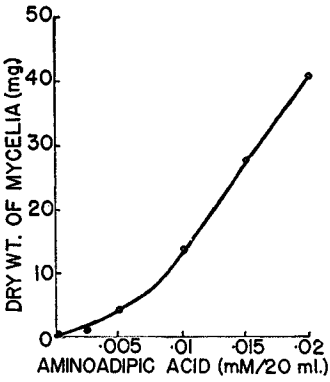

FIG. 1. Growth curve of *Neurospora* mutant 33933 on α-aminoadipic acid. The quantities indicated were added to 20 ml. of minimal medium, autoclaved, inoculated with a suspension of conidia in water, and allowed to grow for 96 hours at 25°.

Since the lysine accounted for only 27.2 per cent of the radioactivity originally present in the α-aminoadipic acid, an attempt was made to determine whether other amino acids had taken up any counts. A starch column was prepared by the Moore and Stein method (6) and 0.15 ml. of hydrolysate put on the column in 1:2:1 solution. The column was placed on an automatic fraction collector adjusted to deliver 0.5 ml. fractions, and a reservoir of 1:2:1 solution was placed above the column. At the aspartic acid locus the reservoir was changed to a 2:1 solution. Odd numbered fractions were analyzed by the Moore and Stein quantitative ninhydrin method (7). At the ninhydrin peaks the even numbered fractions were evaporated to dryness in steel cups and the residue was tested

[2] The bioassays were performed by Dr. S. C. Shen.

for radioactivity. Only in the lysine peak was significant radioactivity found.

DISCUSSION

Since the lysine formed by the mutant had the same level of radioactivity as that provided by the α-aminoadipic acid, it is evident that no alternate pathway for lysine synthesis was being used. This confirms the interpretation of Mitchell and Houlahan previously cited.

The lack of radioactivity in the other amino acids means only that the ϵ-C^{14} of the aminoadipic acid was not used for amino acid synthesis and does not rule out utilization of the rest of the carbon chain for this purpose.

SUMMARY

1. α-Aminoadipic acid is a precursor for lysine synthesis in *Neurospora crassa*.

2. The ϵ-carbon atom of α-aminoadipic acid does not appear in other amino acids.

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