GROWTH OF A *Neurospora crassa* LYSINE AUXOTROPH ON GLUTARIC ACID

C. R. Burke, and H. P. Broquist

Laboratory of Biochemistry, Department of Dairy Science, University of Illinois, Urbana, Ill.

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Some years ago Mattoon and Haight reported (1) that a lysine requiring *Saccharomyces cerevisiae* mutant accumulated glutaric acid, although the relevance of this observation to metabolic events of lysine biosynthesis in yeast has not been clear. Considerable evidence is now at hand, principally from the Murray Strassman Memorial Laboratory, (cf discussion in (2)) for the biogenesis of α-ketoadipic acid, a lysine precursor, via certain reactions analogous to the TCA cycle:

Scheme (a)

Most recently Bhattacharjee et al (3) found that glutarate accumulation in yeast lysine auxotrophs is associated with those mutants that accumulate α-ketoadipic acid (4), and indeed cellfree extracts of such mutants decarboxylate α-ketoadipate (3) yielding glutarate presumably via over all reaction (b):

(b) α-ketoadipate + ½ O_2 ⟶ glutarate + CO_2

Such findings could account for the observations of Mattoon and Haight (1).

In an unrelated study of lysine metabolism in *Penicillium citreoviride*, Tanenbaum and Kaneko hypothesized on the basis of appropriate tracer studies (5), that homoisocitrate, Scheme (a), might be formed alternatively via a condensation of glyoxylate and glutarate:

(c) glyoxylate + glutarate ⟶ homoisocitrate

Neurospora crassa lysine auxotrophs 33933 and STL-7 are known to be blocked at early stages of Scheme (a) above (6). This communication reports that mutant STL-7, but not 33933 gives a slow, submaximal, yet significant growth response to glutarate in lieu of lysine. Furthermore, if STL-7 is cultured on glutarate-1, 5-^{14}C, the lysine of the cell proteins is labelled.

TABLE I

GROWTH RESPONSE OF Neurospora crassa LYSINE AUXOTROPHS TO LYSINE OR GLUTARIC ACID

To a series of 125 ml Erlenmeyer flasks was added 10 ml double strength lysine less medium of Horowitz and Beadle (8), supplements added as indicated in the table, the pH of the flasks adjusted with phosphoric acid as desired, and the volumes brought to 20 ml. Following sterilization the flasks were inoculated with 0.1 ml suspensions of conidiospores and mycelial fragments from agar slants of the N. crassa mutants maintained as described by Doermann (9). Incubation was in stationary culture at 28°C for the periods indicated in the table, following which the mycelial pads were removed, dried overnight at 60° in vacuo and weighed.

pH of Medium	Growth response to supplement, 20 µmoles/ml			
	N. crassa STL-7		N. crassa 33933	
	L-lysine	Glutarate	L-lysine	Glutarate
	Dry weight mycelial mat (mg/10 ml)			
2.5	0 (3 days)	0 (34 days)		
3.0	10 "	1.4 "		
3.5	22 "	1.8 "		
4.0	31 "	3.8 "		
4.5	32 "	4.7* "	78 (4 days)	0 (22 days)
5.0	32 "	2.9 "		
5.5	37 "	0.2 "		
6.0	40 "	0 "		
7.0	35 "	0 "		
8.0	24 "	0 "		
8.5	0 "	0 "		

* A duplicate of this flask was included in this experiment but further supplemented with glutarate -1, 5-^{14}C (0.5 µC) to provide for the experiment of Fig. 1.

RESULTS AND DISCUSSION

The data of Table I indicate that under certain conditions glutaric acid partially meets the growth requirement of N. crassa strain STL-7 for lysine. Thus at pH 4.0 or 4.5, glutarate yields about 1/10 maximum growth after an incubation period 10 times longer than that required for growth on lysine. In other experiments a 10 fold higher concentration of glutarate only doubled the growth of strain STL-7 under similar conditions. Several factors undoubtedly contribute to the slow, submaximal growth response of STL-7 to glutarate especially permeability of this dicarboxylic acid to the cells. For example the amount of glutarate (20 μmoles/ml) yielding 1/10 maximum growth (Table I) is about 200 fold greater than the amount of lysine that would give comparable growth (7). The restricted pH range over which glutarate will give a growth response in lieu of lysine is also emphasized in Table I. Of a number of compounds tested to date, particularly those related to glutarate e. g. α-ketoglutarate and glutaconic acid, none have duplicated the effect of glutarate. However, under conditions which are optimal for a growth response in strain STL-7, glutarate has no effect on growth of strain 33933 in lysine deficient media, Table I.

Unequivocal evidence that glutarate is indeed being utilized for lysine synthesis, rather than exerting some bizarre effect on the growth of strain STL-7, is provided in the experiment of Fig. 1. Lysine of the cell proteins derived from strain STL-7 grown on glutarate-1, 5-^{14}C was radioactive. Experiments aimed at establishing the labelling pattern of ^{14}C in such lysine are in progress and may suggest the manner in which glutarate can participate in lysine anabolism in N. crassa STL-7. It seems unlikely from analogous examples i. e. the pyruvate and α-ketoglutarate dehydrogenase systems, that reaction (b) is reversible. Postulated reaction (c), "homoisocitritase", seems a more likely possibility for the entry of glutarate into the aminoadipate pathway of lysine biosynthesis. In the analogous case of isocitritase, this enzyme is inducible in certain microorganisms and the reaction is reversible.

Mutant STL-7 has been shown to be low (or devoid) of "homocondensing enzyme" activity, reaction (d) (6). Hence, given an exogenous supply of

(d) acetyl CoA + α-ketoglutarate $\longrightarrow$ homocitrate + CoASH

glutarate and sufficient time, the organism apparently provides itself with an alternate point of entry into Scheme (a) other than via reaction (d). Mutant 33933 has homocondensing enzyme and accumulates homocitrate (6), but

until more is known about the relation of glutarate to Scheme (a), the failure of 33933 to respond to glutarate is not clear.

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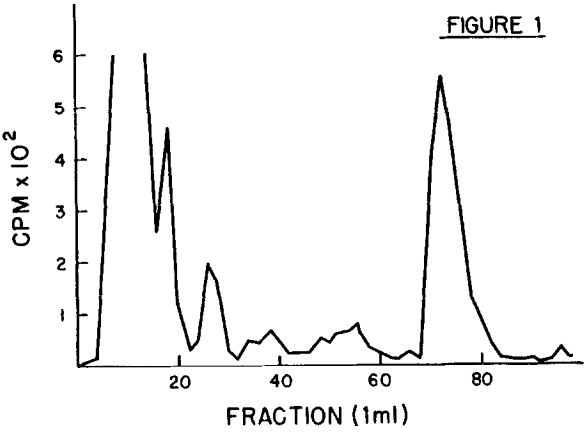

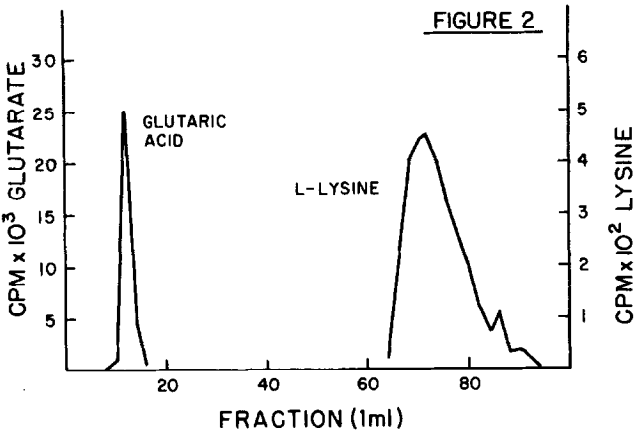

Fig. 1. ELUTION PROFILE OF Neurospora crassa HYDROLYSATE FOLLOWING GROWTH ON GLUTARATE-1, 5-^{14}C. The mycelial mat obtained as described in the footnote of Table 1 was hydrolyzed by autoclaving 5 hrs in 10 ml 6N HCl. Excess HCl was removed by repeated distillation in vacuo. Three mg carrier lysine was added to the hydrolysate and the whole applied in a volume of 1 ml to an Aminex-MS (Bio-Rad Laboratories) cation exchange column (12mm X 80mm) and the column developed following the procedure of Kirkpatrick and Anderson for the isolation of basic amino acids (10). One ml fractions were collected directly into scintillation vials, 15 ml scintillation fluid (0.7% PPO, 7% naphthalene in dioxane) added and the radioactivity determined with a Beckman DPM-100 Scintillation Counter. The data are plotted in Fig. 1.

Fig. 2. ELUTION PROFILE OF AUTHENTIC GLUTARIC ACID-1-5-^{14}C and LYSINE-1-^{14}C FOLLOWING ION EXCHANGE CHROMATOGRAPHY (10).

SUMMARY

Neurospora crassa lysine auxotroph STL-7, blocked early in the aminoadipic acid pathway of lysine biosynthesis, gives a slow, submaximal growth response to a high level of glutaric acid (20 μmoles/ml) in lieu of lysine. When the mutant is grown with glutarate-1, 5-^{14}C, lysine of the cell proteins is labelled. The possible significance of these findings to published observations of a relation of glutarate to lysine metabolism in certain fungi is considered.

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