SACCHAROPINE, A PRODUCT OF LYSINE BREAKDOWN BY MAMMALIAN LIVER

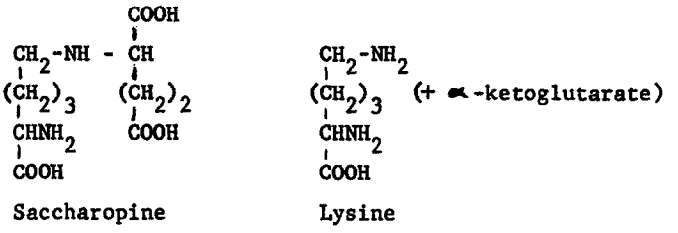
Kazuya Higashino, Kinji Tsukada, and Irving Lieberman

Department of Microbiology, University of Pittsburgh,

School of Medicine, Pittsburgh, Penna. 15213

Received June 16, 1965

Saccharopine (ε-N-(L-glutaryl-2)-L-lysine) (1,2) appears to be the precursor of lysine in the synthetic pathway of Saccharomyces cerevisise according to the reaction (3):



Saccharopine Lysine

Evidence has now been obtained to show that in rat liver: 1) Lysine catabolism occurs largely or only in the mitochondria; 2) α-Ketoglutarate is required for the breakdown of lysine; and 3) Saccharopine is formed during lysine degradation, presumably by a reversal of the reaction that has been described for yeast.

MATERIALS AND METHODS

Uniformly labeled-^{14}C-L-lysine (240 μc/μmole) was from the New England Nuclear Corporation. No radioactive impurities could be detected by chromatography on Dowex 50 or paper. 5-^{14}C-α-Ketoglutaric acid (6.9 μc/μmole) was from the Nuclear-Chicago Corporation. The rats (males, about 80 g) were obtained locally.

Rat liver mitochondria and the other major cell fractions were prepared from homogenates in the usual manner by centrifugation in 0.25M sucrose (4). Lysosomes were isolated according to the procedure of Tappel (5).

Saccharopine, synthesized with a cell-free extract of Fleischmann's yeast, was isolated by chromatography on Dowex 50 and 1. The infrared spectrum[1] and the chromatographic properties of the yeast product were indistinguishable from those of the reported compound (1-3). Saccharopine was measured colorimetrically with ninhydrin (6) and radioactivity measurements were made in a Cab-O-Sil-dioxane mixture in a Packard Tri-Carb liquid scintillation spectrometer.

Table I

Lysine Catabolism by Liver Cell Fractions

The reaction mixtures (2.0 ml), in the main compartments of Thunberg tubes, contained 0.2 ml of 1M Tris-HCl (pH 7.4), 0.06 ml of 0.1M $MgCl_2$, 0.04 ml of 0.01M EDTA, 0.1 ml of 0.1M sodium phosphate (pH 7.4), 0.1 ml of 0.1M α-ketoglutarate, 0.2 ml of boiled liver homogenate, 0.05 ml of ^{14}C-L-lysine (10 μc/ml), and the cell fraction. The stoppers received 0.2 ml of 0.2M KOH. The tubes were evacuated until the reaction mixtures began to foam and they were then incubated at 37° for 30 min. At the end of this time, H_2SO_4 (0.5 ml, 4N) was introduced through the sidearm and the sealed tubes were kept at 60° for 30 min. The radioactivity present in the KOH was estimated as described in "Materials and Methods." The values shown were calculated for the cell fraction obtained from 1 g of wet liver.

Cell fraction	$^{14}CO_2$ formed
	c.p.m.
Homogenate	13,860
Mitochondria	16,160
Postmitochondrial supernatant	350
Nuclei	460
Lysosomes*	400
Postmitochondrial supernatant + mitochondria	13,470
Nuclei + mitochondria	18,300

* The specific activity of acid phosphatase in the lysosomal fraction was three times higher than in the mitochondrial fraction.

1) The authors are indebted to Dr. Gerald L. Carlson, Mellon Institute, for making the infrared analysis.

RESULTS

Lysine Catabolism by Liver Cell Fractions - Under the conditions used to test for lysine breakdown, only the mitochondrial fraction appeared to be active (Table I). As the table shows, the addition of other cell fractions did not enhance the mitochondrial activity.

The Requirement for α-Ketoglutarate - Tested with the mitochondrial fraction, little or no lysine breakdown occurred in the absence of added α-ketoglutarate. As can be seen from the table, none of the other intermediates of the tricarboxylic acid cycle studied was able to satisfy the requirement for α-ketoglutarate.

Table II

The Requirement for α-Ketoglutarate for the Breakdown of Lysine by Liver Mitochondria

Lysine catabolism by liver mitochondria (3.6 mg of protein) was estimated as described in Table I except that in some of the reaction mixtures, as shown, α-ketoglutarate (10 μmoles) was replaced by other compounds (10 μmoles). The values in brackets show the $^{14}CO_2$ formed when α-ketoglutarate, in addition to the test compound, was present in the reaction mixture.

Addition	$^{14}CO_2$ formed
	c.p.m.
None	54
α-Ketoglutarate	2,196
Acetate	44 (1,776)
Pyruvate	76 (2,048)
Oxalacetate	62 (1,768)
Fumarate	52 (2,176)
Malate	42 (1,021)
Succinate	36 (1,178)
Isocitrate	51 (1,540)
Citrate	50 (2,511)
Glutamate	59 (1,695)

Identification of Saccharopine as a Product of Lysine Catabolism by Liver Mitochondria - When liver mitochondria (66 mg of protein) were incubated with 20 μc of ^{14}C-lysine under the conditions described in Table I, 266,600 c.p.m. were recovered as CO_2 and 724,000 c.p.m. were found in an unknown compound that could be adsorbed to both Dowex 50 and 1. The unknown compound was identified as saccharopine by cochromatography with the unlabeled yeast compound (Table III).

Not shown in the table are three additional pieces of evidence for the identity of the mitochondrial product with saccharopine. First, paper chromatography in other solvent systems (n-butanol-acetic acid-water and tertiary-butanol-formic acid-water) gave results similar to the ones shown in Table III. Secondly, heating the radioactive mitochondrial product in 6M HCl at 125°, just as with the yeast compound, yielded a derivative that had the chromatographic properties of pyrosaccharopine (1). The rates of conversion of the mitochondrial product and of yeast saccharopine to the derivative were indistinguishable. Thus, after 15 and 60 min of heating, 27.6 and 42.0%, respectively, of the liver product were converted to the derivative, and with the yeast product, the comparable values were 26.5 and 45.4%. Finally, when mitochondria were incubated with ^{14}C-α-ketoglutarate plus unlabeled lysine, the same radioactive product was isolated as with radioactive lysine plus unlabeled α-ketoglutarate.

DISCUSSION

Lysine catabolism by rat liver appears to be largely or solely a result of the action of enzymes located in the mitochondrion and little or no breakdown occurs in the absence of α-ketoglutarate. Since α-ketoglutarate seems to be specifically required, it seems likely that it serves as a substrate rather than in a secondary role, for example, to provide reduced pyridine nucleotides. It is the specificity and the obligatory nature of the α-ketoglutarate requirement that suggest that saccharopine is a major

Table III

Identification of Saccharopine as a Product of Lysine Catabolism by Liver Mitochondria

A sample of the ^{14}C-labeled mitochondrial product that had been previously purified by chromatography on Dowex 50 and 1 was mixed with yeast saccharopine. The specific activity of the mixture was 1,640 (for chromatography on Dowex 1) or 3,070 c.p.m./μmole (for chromatography on Dowex 50 and paper). Chromatography on Dowex 1 (10X, formate form) was on a column of 6 x 1 cm, elution was with 0.01 M formic acid, and 3 ml fractions were collected. With Dowex 50 (8X, H^+ form), the dimensions of the column were 22 x 0.5 cm, gradient elution (water to 4 M HCl) was used, and 3 ml fractions were collected. With the paper, the sample was streaked along the origin. Chromatography yielded a single band that was located by spraying the edge of the chromatogram with ninhydrin. The unsprayed part of the band was cut into four strips. Saccharopine was estimated with ninhydrin and radioactivity measurements were made as described in "Materials and Methods".

Resin or paper solvent	Fraction or strip number	Saccharopine		
		c.p.m.	μmoles	c.p.m./μmole
Dowex 1	11	75		
	12	405	0.29	1,397
	13	1,800	1.12	1,607
	14	2,886	1.71	1,688
	15	2,805	1.72	1,631
	16	1,626	0.93	1,748
	17	627	0.41	1,529
	18	29		
Dowex 50	25	490	0.13	3,769
	26	1,950	0.66	2,955
	27	4,220	1.38	3,058
	28	5,610	1.87	3,000
	29	4,400	1.44	3,056
	30	2,150	0.95	2,216
	31	610	0.39	1,564
Phenol saturated with water	1	189	0.065	2,908
	2	318	0.104	3,058
	3	237	0.087	2,724
	4	105	0.048	2,188

intermediate on the main pathway of lysine breakdown by rat liver.[2)]

2) The possibility is not excluded, however, that one or more additional pathways exist that have a specific requirement for α-ketoglutarate as an amino acceptor.

If this is so, it would seem that the previous suggestion that pipecolic acid (7) is on the main path is incorrect. Rather, the findings with the intact rat (8) coupled with the accumulation of mitochondrial intermediates tentatively identified as α-aminoadipic acid, α-ketoadipic acid, and glutaric acid, suggest the following pathway for the catabolism of lysine by rat liver: lysine ⟶ saccharopine ⟶ α-aminoadipic-δ-semialdehyde ⟶ α-aminoadipic acid ⟶ α-ketoadipic acid ⟶ glutaric acid. This path is the reverse of the one tentatively proposed for the synthesis of lysine by yeast (3).

REFERENCES

1. Darling, S., and Larsen, P. O., Acta Chem. Scand., 15, 743 (1961).
2. Kjaer, A., and Larsen, P. O., Acta Chem. Scand., 15, 750 (1961).
3. Kuo, M. H., Saunders, P. P., and Broquist, H. P., J. Biol. Chem. 239, 508 (1964).
4. Hogeboom, G. H., Schneider, W. C., and Striebich, M. J., J. Biol. Chem., 196, 111 (1952).
5. Tappel, A. L., Sawant, P. L., and Shibko, S., in A.V.S. de Reuck and M. P. Cameron (Editors), Lysosomes, Ciba Foundation Symposium, Little, Brown and Company, Boston, Mass., 1963.
6. Rosen, H., Arch. Biochem. Biophys., 67, 10 (1957).
7. Rothstein, M., and Miller, L. L., J. Am. Chem. Soc., 75, 4371 (1953).
8. Rothstein, M., and Miller, L. L., J. Biol. Chem., 206, 243 (1954).