METABOLISM OF LYSINE IN RAT LIVER

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It was shown previously that lysine was degraded in the mitochondria in the presence of α-ketoglutarate and, under these conditions, saccharopine accumulated (1). The obligatory requirement of α-ketoglutarate for lysine breakdown as well as for saccharopine formation suggest that lysine is metabolized, in mammalian liver, by a reversal of reaction sequence described for its biosynthesis in yeast (2-6).

The present report describes that saccharopine is further metabolized to α-aminoadipic acid in the mitochondria and that NAD rather than NADP is required in this reaction.

MATERIALS AND METHODS

L-α-Aminoadipic acid was purchased from Calbiochem, and NAD, NADP and FAD from Sigma Chemical Company. ^{14}C-Lysine (uniformly labeled, 136 mC/mmole) was obtained from Radiochemical Centre.

^{14}C-Saccharopine was prepared from ^{14}C-lysine and unlabeled α-ketoglutarate by the action of extracts of bakers' yeast (2, 6). The product was isolated by chromatography over Dowex 50 X8 (H-form) and Dowex 1 X10 (formate-form) as previously described (1). ^{14}C-Saccharopine thus obtained was free from impurity as examined by chromatography on Dowex or paper, and had a specific radioactivity of 1.4 mC/mmole.

Male albino rats were obtained locally and fed on laboratory chow ad

libitum. Mitochondria were obtained from homogenates prepared in 0.25M sucrose-0.001M EDTA by centrifugation in the usual manner (7). Mitochondrial extract was prepared by sonically disrupting the mitochondria in 0.02M Tris-HCl, pH 8.3 (10 kc., 1 min) and by centriguging the sonicate at high speed. The supernatant solution obtained was used as the enzyme solution.

Saccharopine and α-aminoadipate were determined colorimetrically with ninhydrin (8) and radioactivity measurements were made in a dioxane mixture in a Packard Tri-Carb liquid scintillation spectrometer.

RESULTS

With a suspension of mitochondria, little or no saccharopine breakdown occurred. The breakdown, however, was demonstrated by the frozen and thawed preparation or the sonic extract of the mitochondria (Table I). In the experiments described below, the sonic extracts were used.

Table I

Effect of Freezing and Thawing on Saccharopine Degradation

The reaction mixture (1.0 ml) contained 50 μmoles of Tris-HCl, pH 8.3, ^{14}C-saccharopine (11,000 c.p.m.,1.4 mC/mmole), 2.5 μmoles of NAD, 1 μmole of EDTA, 10 μmoles of 2-mercaptoethanol and a mitochondrial suspension or the same frozen and thawed ten times (5.7 mg protein). The mixtures were placed in the main compartments and 0.2 ml of 0.2 N KOH in the stoppers of the Thunberg tubes. The tubes were evacuated and incubated at 37° for 30 min. At the end of the incubation time, 0.25 ml of 2.5N $HClO_4$ was introduced through the side arms, and the sealed tubes were incubated at 60° for 30 min. The radioactivity of CO_2 evolved was determined by counting the KOH fraction. Radioactive saccharopine was estimated after its isolation by chromatography on Dowex 50 X8.

Treatment	^{14}C-Saccharopine disappeared	$^{14}CO_2$ evolved
	c.p.m.	c.p.m.
None	462	16
Freezing and Thawing	5852	396

Table II

Cofactor Requirement for Saccharopine Breakdown by

Sonic Extract of Mitochondria

The complete reaction mixture contained, in a volume of 1.0 ml, 50 μmoles of Tris-HCl, pH 8.3, ^{14}C-saccharopine (15,000 c.p.m., 1.4 mC/mmole), 2.5 μmoles of NAD and a sonic extract of mitochondria which had been dialyzed against 0.02M Tris-HCl, pH 8.3 containing 1mM EDTA and 10mM 2-mercaptoethanol (6.8 mg protein). Incubation was at 37° for 30 min. Saccharopine was estimated after its isolation by Dowex 50 chromatography.

	^{14}C-Saccharopine disappeared
	c.p.m.
Complete	12153
" -NAD	243
" -NAD, +NADP (2.5 μmoles)	4753
" -NAD, +FAD (0.25 μmole)	588

Cofactor Requirements- Table II shows the cofactor requirement for saccharopine degradation. The reaction showed an obligatory requirement for a pyridine nucleotide. NADP was about 30% as active as NAD under the conditions of Table II.

Products of Saccharopine Catabolism- It was previously shown that lysine was degraded by the mitochondria with an evolution of CO_2 when α-ketoglutarate was added (1). Incubation of saccharopine labeled in the lysine moiety with a mitochondrial extract also yielded radioactive CO_2 (Table I). This observation supports the view that lysine is degraded via saccharopine. Chromatography of the reaction mixture on Dowex 50 revealed two radioactive peaks as shown in Fig. 1a. Peak A was shown to be α-aminoadipic acid by rechromatography after the addition of the authentic sample (Fig. 1b). Peak B was the remaining saccharopine. No lysine was formed under these conditions. When the reaction product eluted from Dowex 50 was chromatographed on paper in four solvent

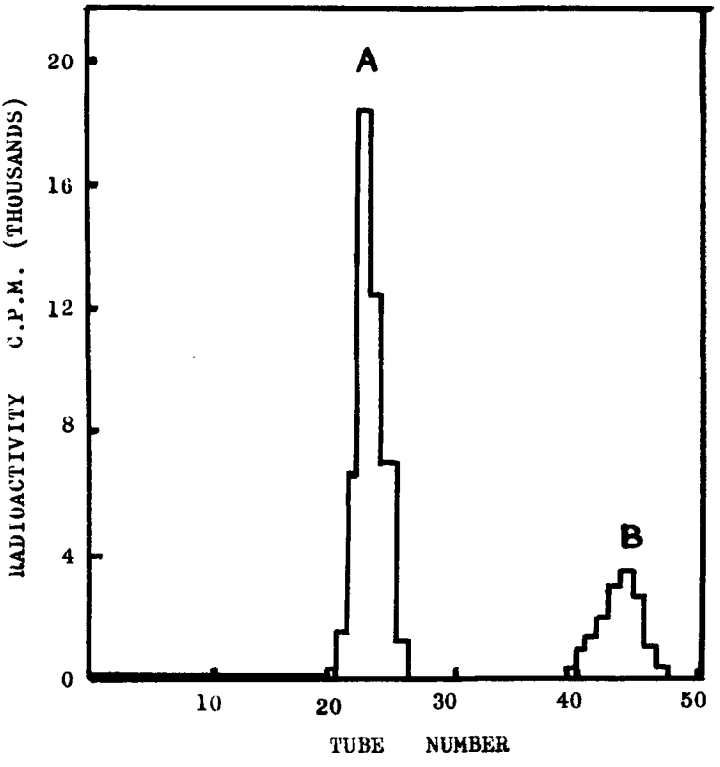

Fig. 1a. Chromatography of the reaction product on Dowex 50 X8. The reaction mixture contained, in a volume of 4.0 ml, 200 μmoles of Tris-HCl, pH 7.4, 4 μmoles of EDTA, 40 μmoles of 2-mercaptoethanol, ^{14}C-saccharopine (1.2 x 10^5 c.p.m., 1.4 mC/mmole) and a sonic extract of mitochondria (15.6 mg protein). After incubation at 37° for 2 hours, the reaction was terminated by the addition of perchloric acid to the final concentration of 5%. The mixture was then neutralized with KOH and the insoluble materials were removed by centrifugation. The supernatant was chromatographed on Dowex 50 X8, H-form (1 x 20 cm). The column was washed with water to remove the radioactive material which was not held on the column. Gradient elution was then applied with H_2O in the mixing vessel (280 ml) and 4 N HCl in the reservoir. Five ml fractions were collected. The figure shows the elution profile from the beginning of gradient elution.

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systems, The radioactivity coincided with the ninhydrin color of α-aminoadipate. The Rf values in pyridine: acetone: ammonia: water (50: 30: 5: 15), isopropanol: formic acid: water (8: 1: 1), phenol saturated with water, and tert-butanol: formic acid: water (70: 15: 15) were 0.18, 0.46, 0.27 and 0.54, respectively. Furthermore, repeated crystallization after the addition of unlabeled α-aminoadipate yielded a constant specific activity (Table III). This provides further evidence for the identity of the product with α-aminoadipic acid.

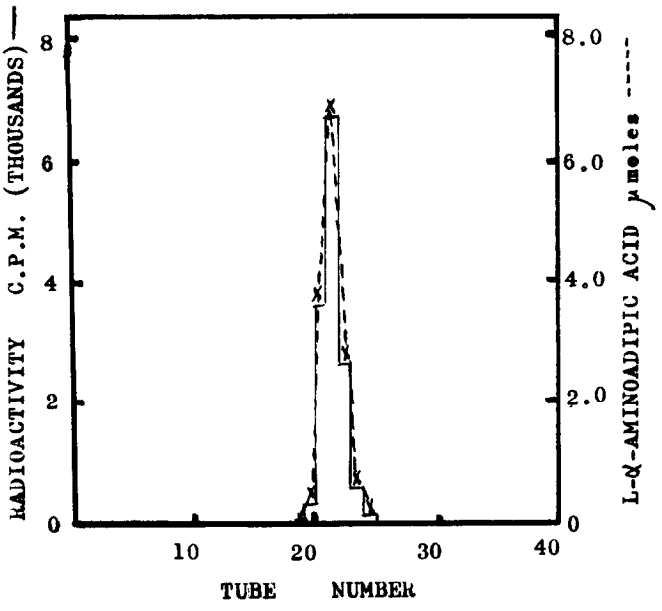

Fig. 1b. Co-chromatography of the reaction product with L-α-aminoadipic acid. Peak A of Fig. 1a was taken to dryness and the residue dissolved in H_2O. To an aliquot containing 1,500 c.p.m. were added 15 μmoles of L-α-aminoadipate. Chromatography was on Dowex 50 X8 under the same conditions as in Fig. 1a.

Table III

Specific Radioactivities of Reaction Product after

Repeated Crystallizations

To an aliquot of the degradation product of ^{14}C-saccharopine (Peak A, Fig. 1a) containing about 18,000 c.p.m. were added 100 mg of unlabeled L-α-aminoadipic acid. Recrystallization was from ethanol-H_2O mixture. At each crystallization, the compound was dried over P_2O_5 and 5 mg portions were used for radioactivity measurements.

Crystallization	mg	Specific activity of α-aminoadipic acid
		c.p.m./mg
1st	49.3	169
2nd	16.2	165
3rd	5.5	164

DISCUSSION

Lysine breakdown was shown to occur in liver mitochondria only when supplemented with α-ketoglutarate. This fact and the obligatory requirement of α-ketoglutarate for saccharopine formation suggest that lysine catabolism proceeds by a reversal of the pathway of its biosynthesis in Saccharomyces and Neurospora (2-6). The possibility exists, however, that although α-ketoglutarate participates somewhere inthe process of lysine breakdown, saccharopine formation is only a side reaction.

The data described in this communication present evidence that saccharopine is further metabolized to α-aminoadipate in liver mitochondria. Although the immediate products of saccharopine catabolism would be α-aminoadipic-δ-semialdehyde and glutamate, neither the aldehyde nor its cyclization product (Δ^1-piperideine-6-carboxylic acid) was detected under the conditions used in this investigation.

To understand the importance of the saccharopine pathway in mammalian metabolism of lysine, it will be necessary to know the comparable rates at which lysine and saccharopine are degraded in mitochondria. However, the determination of these rates is rendered difficult by the fact that saccharopine does not penetrate the mitochondrial membrane (Table I) and conversely saccharopine formation is not effected ny disrupted mitochondria.

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