

## Biosynthesis and Degradation of Saccharopine, an Intermediate of Lysine Metabolism

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Lysine-2-oxoglutarate reductase was prepared from ox liver and its characteristics were examined. Its activity was totally inhibited in the presence of  $\text{NH}_4\text{Cl}$ . Under conditions that inhibit saccharopine formation, and in the presence of  $\text{NADP}^+$ , ox liver mitochondria were found to catalyse the hydrolysis of saccharopine to lysine and  $\alpha$ -oxoglutarate. The enzyme involved was named saccharopine oxidoreductase. It was partially purified and separated from lysine-oxoglutarate reductase. Comparison of the properties of these two enzymes showed that saccharopine degradation was stimulated under conditions that inhibit its formation. The effect of pH, various cofactors and stability during incubation confirm that saccharopine biosynthesis from, and degradation to, lysine are catalysed by two distinct enzymes.

Since the concept of essential amino acids was first described (Osborne & Mendel, 1914), lysine has been known to be necessary for mammalian growth and maintenance of body weight.

The unique irreversibility of the oxidative transamination of lysine was discussed by Weissman & Schoenheimer (1941). Experiments with rats indicated that the ultimate fate of lysine N is excretion as urea and  $\text{NH}_3$  (Neuberger & Sanger, 1944). The intermediary metabolism of lysine has not yet been established, although the initial degradative steps appear to involve two distinct pathways. One, via pipecolic acid, involves the removal of the  $\alpha$ -amino group of lysine (Rothstein & Miller, 1954). The second involves the intermediate formation of saccharopine, followed by the removal of the terminal amino group (Higashino *et al.*, 1965).

The current study arose from our interest in a case of saccharopinuria reported by Carson *et al.* (1968). The patient excreted an excess amount of lysine and a large quantity of saccharopine in her urine. Increased amounts of lysine and saccharopine were also observed in the patient's plasma and cerebrospinal fluid. Enzyme studies in the skin fibroblasts grown from the patient and from normal individuals revealed that the patient's fibroblasts were completely lacking in activity of saccharopine dehydrogenase which catalyses the cleavage of saccharopine to  $\alpha$ -aminoadipate  $\delta$ -semialdehyde and glutamate (Fellows, 1972). When compared with normal control subjects the patient's fibroblasts showed a decreased activity of lysine-oxoglutarate reductase which catalyses the formation of saccharopine from lysine and  $\alpha$ -oxoglutarate. This may account for the high concentration of lysine

observed in saccharopinuria. Alternatively it may be explained by the reversibility of the reaction from lysine to saccharopine. The present paper describes the identification and characterization, in ox liver, of an enzyme capable of hydrolysing saccharopine to lysine and  $\alpha$ -oxoglutarate. This enzyme, which has been previously reported (Wang & Lewis, 1972), has been named saccharopine oxidoreductase.

### Experimental

#### Materials

Ox livers were collected at the local abattoir immediately after slaughter and transported to the laboratory in ice.

L-[U- $^{14}\text{C}$ ]Lysine hydrochloride was obtained from The Radiochemical Centre, Amersham, Bucks., U.K. All other chemicals and reagents were of the highest purity commercially available.

#### Methods

*Desalting technique.* Cation-exchange column chromatography was used for desalting the deproteinized incubation media and amino acid fractions. A column (1 cm diam.  $\times$  25 cm) was specially made for the purpose of desalting small quantities of solutions. A 100 ml conical flask was joined to the top of the column and a tap at the bottom. Dowex 50W (X8 resin;  $\text{H}^+$  form; 200-400 mesh) was used. The wet resin was packed into the column giving a bed volume of 5 cm. The column was precycled twice with 2M- $\text{NH}_3$  (20 ml), deionized water (50 ml) followed by 1M-HCl (20 ml) and deionized water (50 ml).

The sample to be desalted was acidified to pH 2.0 and loaded on the resin. After the sample had passed

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through the resin it was washed with deionized water (50ml) and then eluted with 2M-NH<sub>3</sub> (20ml). The first 4ml of eluate (pH4-5) was discarded. The next 10ml (>pH10) contained amino acids and was collected. This eluate was dried at 50°C and the residue was redissolved in water. This latter solution represented the desalted aqueous amino acid mixture, which was then analysed by either two-dimensional chromatography or high-voltage electrophoresis.

*Two-dimensional paper chromatography.* All samples were desalted before paper chromatography. Whatman chromatography paper no. 1 (20cm × 20cm) was used. The papers were chromatographed first in butan-1-ol-pyridine-water (1:1:1, by vol.) for 16h. After drying, the papers were then chromatographed at a 90° angle to the previous direction for 7h in phenol-water (2:1, w/v). Aq. NH<sub>3</sub> (1ml, sp.gr. 0.880) added to the chromatography tank before the run facilitated separation of basic and neutral amino acids.

*High-voltage electrophoresis.* A Shandon high-voltage electrophoresis apparatus, Model L24, was used for all separations. All samples were desalted before electrophoresis. Whatman 3MM paper was used. Electrophoresis was conducted at 3000V for 90min in sodium barbitone buffer (0.05M, pH8.2). Under these conditions lysine and other basic amino acids travelled to the cathode and saccharopine, glutamate and other acidic amino acids to the anode.

*Preparation and isolation of saccharopine.* L-[lysyl-U-<sup>14</sup>C]Saccharopine {ε-N-(glutaryl-2)-L-[U-<sup>14</sup>C]-lysine} was synthesized enzymically by using a partially purified lysine-oxoglutarate reductase prepared from ox liver by the method of Hutzler & Dancis (1968). The incubation mixture contained: L-[U-<sup>14</sup>C]-lysine hydrochloride (100μCi); L-lysine hydrochloride (2μmol); α-oxoglutarate (4μmol); NADPH (4μmol); potassium phosphate buffer, pH7.0 (50μmol); enzyme extract and water to a final volume of 1ml. Incubation was for 90min at 30°C and under a constant flow of N<sub>2</sub>. The reaction was terminated by the addition of sulphosalicylic acid (50mg) which precipitated the protein in the mixture. The deproteinized supernatant was applied to a Technicon amino acid auto-analyser (the buffer gradient used here was that of Wang & Carson, 1968) and the appropriate fractions were collected by a BTL fraction collector. L-[lysyl-U-<sup>14</sup>C]Saccharopine and the unchanged L-[U-<sup>14</sup>C]lysine were collected separately. The L-[lysyl-U-<sup>14</sup>C]saccharopine fraction was desalted by a column of Dowex 50W [X8]. The desalted aqueous L-[lysyl-U-<sup>14</sup>C]saccharopine solution was stored at -20°C until required. The product, which was detected by X-ray radioautography, was shown to behave homogeneously by both high-voltage electrophoresis and two-dimensional chromatography. The total yield was 40μCi. The unchanged

L-[U-<sup>14</sup>C]lysine was purified in the same manner and yielded 43μCi of pure lysine.

L-Saccharopine was isolated from the urine of the patient with saccharopinuria. The method used was modified from that of Darling & Larsen (1961). Urine (10 litres) was freeze-dried and redissolved in a minimum amount of water. The solution was acidified with 4M-HCl to pH2.0 and then centrifuged to remove the precipitate. The clear supernatant was desalted by a column (5cm × 70cm) of Dowex 50W (X8). The desalted aqueous amino acids solution was passed through an anion-exchange column (Amberlite IR-4B, 5cm × 70cm) prepared by the method of Darling & Larsen (1961). The column was first washed with 1 litre of CO<sub>2</sub>-free water. The eluate contained neutral and basic amino acids and was discarded. The acidic amino acids, including saccharopine, were eluted with 3M-HCl. The eluate was evaporated to dryness at 50°C and redissolved in a minimum amount of water. The evaporation procedure was repeated three times to remove the HCl in the amino acid mixture. The amino acid mixture was adjusted to pH2.5 with 4M-HCl and 8vols. of ethanol were added. Crystallization occurred almost immediately. The suspension was kept at 4°C for 72h. The precipitate was filtered and washed, first with ethanol-0.01M-HCl solution (8:1, v/v) and then with acetone. The product was redissolved in a minimum amount of 0.01M-HCl and decolorized with activated acid-washed charcoal powder. The collection of filtrate and washings was evaporated to a small volume and 8vols. of ethanol were added. The product, which contained glutamate as contaminant, was further purified by additional crystallization.

*Purification of saccharopine oxidoreductase.* All procedures were carried out at 0-4°C.

Crude homogenate. Ox liver (200g) was minced with scissors to obtain small fragments and rinsed a few times with cold buffered iso-osmotic sucrose solution [sucrose (0.25M); EDTA (1mM) and Tris-HCl, pH7.4 (5mM)] to remove any contaminating blood. The liver was further minced in an Ato-Mix laboratory blender (MSE) with 5vols. of the buffered sucrose solution for 30s at a low speed. This initial homogenate was then homogenized in a Potter-Elvehjem homogenizer for five passes of a Teflon pestle. A small fraction of this final homogenate was sonicated (5 × 20s) in an ultrasonic disintegrator (100W, MSE), giving fraction 1, which was tested for saccharopine oxidoreductase activity.

Mitochondrial fraction. Mitochondria were isolated from the crude homogenate by the method of Chappell & Hansford (1969). The mitochondria were suspended in 100ml of a standard buffer solution [Tris-HCl, pH7.0 (0.1M); 2-mercaptoethanol (1mM) and EDTA (1mM)]. The suspension was sonicated (5 × 20s) and the disrupted mitochondrial suspension was stirred for 30min. It was then centrifuged for

20 min at 25000g. The supernatant (fraction 2) was tested for saccharopine oxidoreductase activity.

(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> fractionation. Fraction 2 was diluted to 125 ml with standard buffer solution to contain 10 mg of protein/ml. Solid (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> was added slowly, with constant stirring, to a concentration of 30% saturation. Stirring was continued for 30 min. The precipitate was removed by centrifugation and redissolved in a standard buffer solution and dialysed against 100 vol. of the standard buffer for 6 h (precipitate 1). Similarly the (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> concentration in the supernatant was brought to 40% and then 50% saturation. The precipitates obtained at 40% and 50%-(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> saturation were redissolved in standard buffer solution and dialysed to give precipitates 2 and 3 respectively. The supernatant from 50% saturation of (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> was also dialysed, giving the soluble fraction. Precipitates 1, 2, 3 and the soluble fraction were all tested for saccharopine oxidoreductase activity.

*Protein determination.* Protein was measured by the method of Lowry *et al.* (1951) with crystalline bovine serum albumin as standard.

*Standard assay conditions for lysine-oxoglutarate reductase.* The assay conditions were based on those used by Hutzler & Dancis (1968). The incubation medium contained in 0.5 ml: L-lysine hydrochloride (1.0 μmol); L-[U-<sup>14</sup>C]lysine (0.1 μCi); α-oxoglutarate (2.0 μmol); NADPH (2.0 μmol); potassium phosphate buffer, pH 7.0 (50.0 μmol); enzyme extract (1.0 mg of protein). The incubation was for 30 min at 30°C under a constant flow of N<sub>2</sub>. The reaction was terminated by the addition of sulphosalicylic acid (50 mg) and the precipitated protein was removed by centrifugation. L-Saccharopine (1.0 μmol) was then added to the supernatant as carrier and the mixture was desalted on Dowex 50W (X8). The radioactive saccharopine was separated by means of high-voltage electrophoresis and the radioactivity was determined by liquid-scintillation counting.

*Standard assay conditions for saccharopine oxidoreductase.* The incubation medium contained in 0.5 ml: L-saccharopine (1.0 μmol); L-[lysyl-U-<sup>14</sup>C]-saccharopine (0.2 μCi); NADP<sup>+</sup> (2.0 μmol); NH<sub>4</sub>Cl (25 μmol); Tris-HCl buffer, pH 9.0 (50 μmol) and enzyme extract (1.0 mg of protein). Incubation was for 1 h at 30°C and the reaction was terminated by the addition of sulphosalicylic acid (50 mg). L-Lysine (1.0 μmol) was added as carrier into the deproteinized medium. Amino acid analysis procedures of the incubation medium and the method for determination of L-[U-<sup>14</sup>C]lysine content were the same as those used for radioactive saccharopine.

*Liquid-scintillation counting.* The amino acids separated by either high-voltage electrophoresis or two-dimensional paper chromatography were located by spraying the paper with ninhydrin (0.1% in acetone, w/v). The spots were then cut out and shaken

in 2 ml of 50% methanol solution at room temperature for 4 h. Bray's (1960) scintillation fluid (15 ml) was added to the extract and counted for radioactivity in a Packard Tri-Carb liquid-scintillation counter after a 4 h equilibration period in the counting chamber.

Quench correction was made by the method of Bell (1968) by using an automatic external standard.

## Results and Discussion

Saccharopine is synthesized from lysine and α-oxoglutarate in mammalian liver. Lysine-oxoglutarate reductase prepared from ox liver by the method of Hutzler & Dancis (1968) was found to have an activity of 21000 pmol/min per mg of protein. In studies on the ability of this preparation to catalyse the hydrolysis of saccharopine to lysine and α-oxoglutarate, the preparation was incubated with saccharopine over a pH range 6.0-10.0. In addition, the possible role of cofactors for the enzyme was studied. NADP<sup>+</sup>, NAD<sup>+</sup>, ATP, MgCl<sub>2</sub> and pyridoxal 5'-phosphate were incubated separately or in combination, with the enzyme preparation in the presence of saccharopine. All of these incubations were carried out at 30°C for 1 h. On no occasion was lysine formed from saccharopine. Repetition of these studies over a temperature range (20-37°C) also failed to give any indication that lysine-oxoglutarate reductase catalyses the formation of lysine from saccharopine.

The activity of lysine-oxoglutarate reductase must therefore be inhibited in any attempt to establish the presence, in liver homogenates, of a second enzyme capable of catalysing the formation of lysine from saccharopine. Further studies showed that synthesis of saccharopine by liver homogenate was completely inhibited by NH<sub>4</sub>Cl (50 mM). Lysine was not formed when liver homogenates were incubated with saccharopine and NH<sub>4</sub>Cl (50 mM). However, when various cofactors (NAD<sup>+</sup>, NADP<sup>+</sup>, ATP, MgCl<sub>2</sub> and pyridoxal 5'-phosphate) were included in the incubation mixture it was found that, in the presence of exogenous NADP<sup>+</sup>, a small amount of lysine was formed from saccharopine by liver homogenate. The maximum formation of lysine by liver homogenate was found to occur around pH 9.0.

### *Cellular distribution of saccharopine oxidoreductase*

Saccharopine oxidoreductase activity was shown to be largely located in the mitochondrial fraction (fraction 3, Table 1) of ox liver. However, an appreciable amount of enzyme activity was distributed in other fractions of the cell. An examination of the techniques used in the fractionation of subcellular components suggested that the activities in fractions 2 and 4 are probably due to mitochondrial contamination of these fractions. Because of its comparatively

Table 1. Cellular distribution of saccharopine oxidoreductase

Assay conditions were those described in 'Methods'. Enzyme activity was estimated by the calculation of radioactivity of L-[U-<sup>14</sup>C]lysine formed from L-[*lysyl*-U-<sup>14</sup>C]saccharopine. The total wet weight of liver fractionated was 10g.

Cellular fraction	Total protein (mg)	Total activity (nmol of lysine formed/min)
Fraction 1 (whole liver homogenate)	640	34.9
Fraction 2 (precipitated at 700g)	180	2.8
Fraction 3 (mitochondria, precipitated at 9000g)	98	39.2
Fraction 4 (post-mitochondrial supernatant)	325	7.3

fibrous texture, ox liver had to be minced before cell disruption with a Potter-Elvehjem homogenizer, and this mincing may have caused some damage to the mitochondria. This would lead to the contamination by mitochondrial protein in the soluble fraction (fraction 4). The enzyme activity found in fraction 2 may be due to incomplete disruption of the liver cells during homogenization. Refinement of the technique of differential centrifugation led to a considerable decrease in the total yield of mitochondria. In general, the total protein recovered from the cell fractions was 95% of that in the whole liver homogenate.

#### Optimum pH

The effect of pH on the initial reaction velocity of saccharopine oxidoreductase was studied in the range of pH values between 7.0 and 10.0 (Fig. 1) and the maximum activity was at pH 9.2.

The conversion of saccharopine into  $\alpha$ -aminoadipate  $\delta$ -semialdehyde and glutamate by saccharopine dehydrogenase requires an alkaline pH (Hutzler & Dancis, 1970). The present findings show the optimum pH of saccharopine oxidoreductase (9.2) to be close to that of saccharopine dehydrogenase (8.8). Thus saccharopine oxidoreductase and saccharopine dehydrogenase, enzymes which are of prime significance in the metabolism of saccharopine, both exhibit maximum activity under alkaline conditions. These findings contrast sharply with the optimum pH of 7.0 reported by Hutzler & Dancis (1968) for lysine-oxoglutarate reductase, which catalyses the formation of saccharopine from lysine and  $\alpha$ -oxoglutarate. All three enzymes involved in saccharopine metabolism are active in ox and human liver mitochondria (Fellows, 1972). Therefore under physiological conditions the synthesis of saccharopine, the initial step of lysine degradation, would presumably require a neutral pH. Further degradation of saccharopine, however, requires alkaline conditions. This suggests

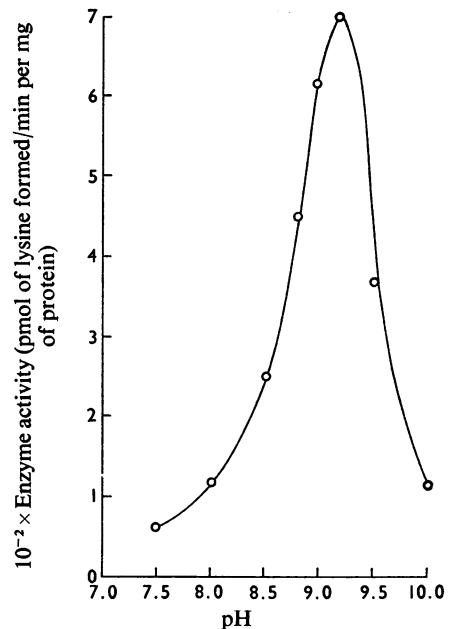


Fig. 1. Effect of pH on saccharopine oxidoreductase activity

The standard assay conditions described in the Experimental section were used except for the variation of buffer pH. Mitochondrial fraction was used as the enzyme source.

that there may be a localization of H<sup>+</sup> ions within the mitochondrion.

#### Effect of NH<sub>4</sub>Cl

Saccharopine oxidoreductase activity was stimulated *in vitro* when NH<sub>4</sub>Cl was included in the incu-

bation medium. Maximum stimulation by  $\text{NH}_4\text{Cl}$  was observed at a concentration of 80 mM (Fig. 2) under the incubation conditions described in 'Methods'. At a concentration of 50 mM in the incubation medium  $\text{NH}_4\text{Cl}$  completely inhibits lysine-oxoglutarate reductase activity. The present study (Fig. 2) showed that the activity of saccharopine oxidoreductase in incubation mixtures containing 50 mM  $\text{NH}_4\text{Cl}$  was 60% of that observed at an  $\text{NH}_4\text{Cl}$  concentration of 80 mM.

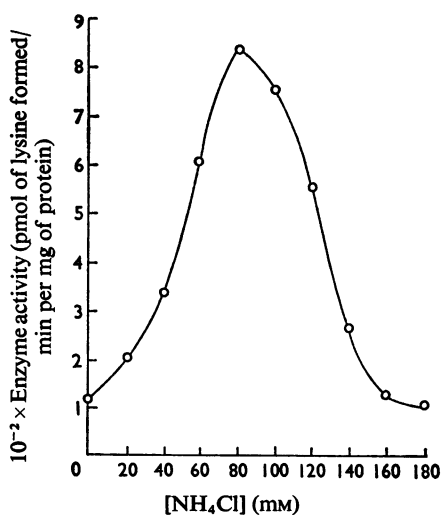


Fig. 2. Effect of  $\text{NH}_4\text{Cl}$  on saccharopine oxidoreductase activity

Assays were conducted in the standard conditions except that the pH of buffer was 9.2 and that the indicated concentrations of  $\text{NH}_4\text{Cl}$  were used. Mitochondrial fraction was used as the enzyme source.

#### Purification of saccharopine oxidoreductase

When liver mitochondrial protein was fractionated with  $(\text{NH}_4)_2\text{SO}_4$  the protein precipitated between 0–30% saturation and above 40% was found to have very low saccharopine oxidoreductase activity. Some 85% of the activity obtained from  $(\text{NH}_4)_2\text{SO}_4$  fractionation of liver mitochondrial protein (Table 2) was found between a saturation of 30–40%, and an appreciable purification of saccharopine oxidoreductase was obtained. Ion-exchange column chromatography of this fraction showed that the enzyme was adsorbed by anion exchangers (either Sephadex A-50 or Whatman DE52) at pH 7.5. Various gradient elution systems were attempted but the total activity recovered was never greater than 4%, and the procedure was therefore considered to be of no practical value.

Initial attempts to purify saccharopine oxidoreductase by gel filtration (a column of 2.5 cm × 70 cm) on Sephadex (G-100, G-200) were unsuccessful. It was therefore decided to investigate the properties of saccharopine oxidoreductase by examining its activity in liver mitochondria and in an  $(\text{NH}_4)_2\text{SO}_4$  precipitate (fraction 3, Table 2) from mitochondrial protein.

Although the activity of saccharopine oxidoreductase in mitochondrial extracts is stable for several months when stored at  $-25^\circ\text{C}$ , that of the partially purified enzyme is less stable and loses 50% of its activity after 1 week at  $-25^\circ\text{C}$ .

#### Studies of partially purified saccharopine oxidoreductase

The effects of pH and of  $\text{NH}_4\text{Cl}$  on the partially purified enzyme were identical with those observed in mitochondria.

The enzyme was almost completely inactive in catalysing the formation of lysine from saccharopine

Table 2. Purification of saccharopine oxidoreductase

The incubation medium contained in 0.5 ml: L-saccharopine (1.0  $\mu\text{mol}$ ); L-[*lysyl*-U- $^{14}\text{C}$ ]saccharopine (0.2  $\mu\text{Ci}$ ); NADP (2.0  $\mu\text{mol}$ );  $\text{NH}_4\text{Cl}$  (40.0  $\mu\text{mol}$ ); Tris-HCl buffer, pH 9.2 (50.0  $\mu\text{mol}$ ), and enzyme extract (1 mg of protein). Enzyme activity was estimated by the calculation of radioactivity of L-[U- $^{14}\text{C}$ ]lysine formed from L-[*lysyl*-U- $^{14}\text{C}$ ]saccharopine.

Fraction	Total protein (mg)	Total activity (nmol of lysine formed/min)	Sp. activity (pmol of lysine formed/min per mg of protein)	Purification (fold)
Fraction 1 (homogenate)	12260	1250	102	1
Fraction 2 (mitochondria)	1250	937	750	7.3
Fraction 3 [30–40% -satd. $(\text{NH}_4)_2\text{SO}_4$ ]	690	2070	3000	29.4

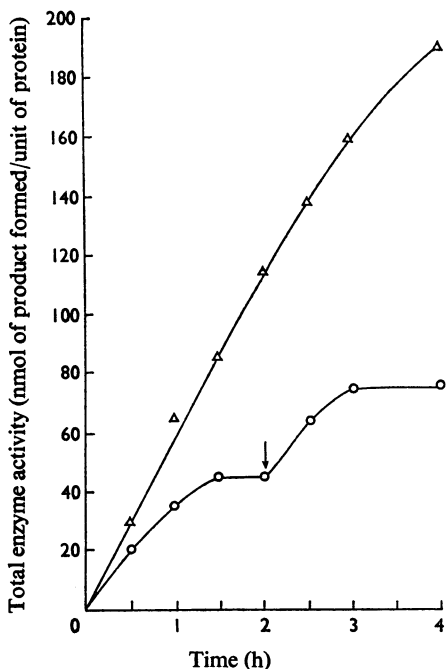


Fig. 3. Relative stabilities of saccharopine oxidoreductase and lysine-oxoglutarate reductase during incubation

Saccharopine oxidoreductase assay conditions were the same as those described in Table 2 and mitochondrial fraction (1 mg of protein) was used as the enzyme source. Lysine-oxoglutarate reductase assay conditions were those described in the Experimental section and mitochondrial fraction (250  $\mu$ g of protein) was used as the enzyme source.  $\circ$ , Saccharopine oxidoreductase;  $\Delta$ , lysine-oxoglutarate reductase. The arrow indicates when fresh enzyme was introduced.

when  $\text{NADP}^+$  (4 mM) was omitted from the study *in vitro*. When  $\text{NADP}^+$  was substituted by either  $\text{NAD}^+$  (4 mM) or ATP (4 mM) and  $\text{MgCl}_2$  (4 mM) negligible stimulation of enzyme activity was observed. In yeast and *Neurospora*,  $\text{NAD}^+$  is required by the enzymes that catalyse the synthesis of lysine from saccharopine (Saunders & Broquist, 1966) in contrast with the requirement for  $\text{NADP}^+$  by saccharopine oxidoreductase in ox liver.

The activity of saccharopine oxidoreductase was almost completely inhibited by *p*-chloromercuribenzoate (0.1 mM) and  $\text{HgCl}_2$  (1.0 mM), but in the presence of an excess of 2-mercaptoethanol (3.0 mM) the inhibition of the enzyme by mercury compounds was less effective. These observations suggest that the active site of the enzyme involves  $-\text{SH}$  groups. Saccharopine oxidoreductase is therefore presumed to be a thiol enzyme.

#### Comparison of saccharopine oxidoreductase and lysine-oxoglutarate reductase

Studies on the relative stabilities of saccharopine oxidoreductase and lysine-oxoglutarate reductase during incubation showed that saccharopine oxidoreductase was stable for 90 min at  $30^\circ\text{C}$ . After 2 h incubation at  $30^\circ\text{C}$  with saccharopine, the initial reaction rate could be restored by introducing into the incubation mixture fresh enzyme that had previously been stored at  $-25^\circ\text{C}$  (Fig. 3). In contrast, lysine-oxoglutarate reductase present in the same preparation, was found to be stable for a period of 4 h at  $30^\circ\text{C}$  (Fig. 3).

$(\text{NH}_4)_2\text{SO}_4$  fractionation of ox liver mitochondrial extracts resulted in a partial resolution of saccharopine oxidoreductase and lysine-oxoglutarate reductase (Table 3). Saccharopine oxidoreductase activity was almost exclusively present in the fraction precipitated between 30–40%  $(\text{NH}_4)_2\text{SO}_4$  saturation.

Table 3. Distribution of saccharopine oxidoreductase and lysine-oxoglutarate reductase activities in various  $(\text{NH}_4)_2\text{SO}_4$  fractions

The assay conditions for lysine-oxoglutarate reductase were those described in 'Methods' and those for saccharopine oxidoreductase were the same as described in Table 2. Enzyme activities were measured by the determination of radioactive products formed by the corresponding enzyme.

$(\text{NH}_4)_2\text{SO}_4$ fraction	Distribution in fractions (%)	
	Lysine-oxoglutarate reductase	Saccharopine oxidoreductase
0–30% saturation	8.4	5.0
30–40% saturation	30.2	85.0
40–50% saturation	51.3	6.0
Soluble fraction	10.1	4.0

Lysine-oxoglutarate reductase activity was present in each fraction, but was considerably higher in the fraction between 40–50%-(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> saturation. These findings indicate that saccharopine oxidoreductase and lysine-oxoglutarate reductase are two distinct proteins.

The synthesis and degradation of saccharopine therefore involves three distinct enzymes. The pathway of lysine degradation via saccharopine and  $\alpha$ -aminoadipate has been confirmed by studies of inherited defects of lysine metabolism (Dancis *et al.*, 1969; Fellows, 1972) in the human. A deficiency of saccharopine dehydrogenase results in the accumulation of both saccharopine and lysine in the patient's body fluids and gives rise to saccharopinuria. The concomitant hyperlysinaemia observed in the patient with saccharopinuria may be explained by stimulation of the hydrolysis of saccharopine to lysine. The present work has confirmed that this pathway, catalysed by saccharopine oxidoreductase, operates in normal ox liver. The activity of the enzyme is, however, considerably higher than that found in the human and other mammals, as reported in the next paper (Fellows & Lewis, 1973).

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