Saccharopine, an Intermediate of the Aminoadipic Acid Pathway of Lysine Biosynthesis

IV. SACCHAROPINE DEHYDROGENASE*

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SUMMARY

The ε-amino group of lysine arises in yeast and Neurospora from glutamate in a unique transamination between α-aminoadipic δ-semialdehyde and glutamate involving the stable intermediate saccharopine.

Aminoadipic semialdehyde + glutamate + NADPH → saccharopine + NADP+ + H2O

The enzyme catalyzing the terminal reaction, saccharopine dehydrogenase (ε-N-(L-glutaryl-2)-L-lysine:NAD oxidoreductase (L-lysine forming)) has been purified and shown to be a sulfhydryl enzyme having an approximate molecular weight of 49,000. Two distinct pH optima (approximately 10 and 7) exist for the forward and reverse directions of the enzyme-catalyzed reaction. The enzyme is stabilized in the presence of relatively high salt concentrations and has been shown to display a high degree of specificity with respect to the coenzyme and substrates. The Michaelis constants for lysine, oxoglutarate, and reduced nicotinamide adenine dinucleotide have been found to be 1.2 × 10⁻³ M, 4.4 × 10⁻⁴ M, and 4.6 × 10⁻⁵ M, respectively.

α-Aminoadipic δ-semialdehyde + glutamate + NADPH + H⁺ → saccharopine + NADP⁺ + H2O

In the forward direction, a reductive condensation between the amino group of glutamate and the carbonyl function of aminoadipic semialdehyde takes place to give saccharopine, an intermediate of the aminoadipic acid pathway of lysine biosynthesis (2, 3). This paper is concerned with the purification and properties of a second enzyme, saccharopine dehydrogenase (ε-N-(L-glutaryl-2)-L-lysine:NAD oxido-reductase (L-lysine forming)) that oxidatively cleaves saccharopine to yield lysine.

Saccharopine + NAD⁺ + H2O → lysine + 2-oxoglutarate + NADH + H⁺

Reaction 2 is also fully reversible and the forward reaction, of concern in lysine biosynthesis, can be shown in vitro at an alkaline pH. The combined action of these two enzymes results in a seemingly classical transamination reaction (sum of Reactions 1 and 2).

EXPERIMENTAL PROCEDURE

Materials—Saccharopine was a generous gift from Dr. P. O. Larson. Saccharopine-¹⁴C was obtained by the procedure previously described (6). Dr. W. K. Paik generously provided ε-N-methyllysine; 2-oxoadipic acid was made available by Dr. M. Bullock. All of the other materials were obtained from commercial sources.

Enzyme Assays—Two methods of assaying enzyme activity were devised; the first employed radioactive lysine while the second, and more generally used, made use of the pyridine nucleotide involvement in the reaction. In each case the reverse reaction, the formation of saccharopine, was carried out.

The incubation mixtures for the assay employing ¹⁴C contained 0.75 μmol of L-lysine-HCl, 0.05 μC of ε-N-methyllysine, 2-oxoadipic acid was made available by Dr. M. Bullock. All of the other materials were obtained from commercial sources.

In each case the reverse reaction, the formation of saccharopine, was carried out.

The preceding paper (1) established that an enzyme in yeast aminoadipic semialdehyde-glutamate reductase (ε-N-(L-glutaryl-2)-L-lysine : NAD(P) oxidoreductase (2-aminoadipate-semialdehyde forming)) catalyzes the following reversible reaction.

ε-N-(glutaryl-2)-L-lysine + NAD⁺ + H⁺ → aminoadipic δ-semialdehyde + glutamate + NADPH

The preceding paper (1) established that an enzyme in yeast aminoadipic semialdehyde-glutamate reductase (ε-N-(L-glutaryl-2)-L-lysine : NAD(P) oxidoreductase (2-aminoadipate-semialdehyde forming)) catalyzes the following reversible reaction.
make the final volume to 0.5 ml. The reaction mixtures were incubated at 25°C for 1 to 2 hours, followed by heating in a boiling water bath for 2 min to denature protein. The solutions were centrifuged to remove the flocculent precipitate and 20-μl samples of the supernatant solutions were applied to paper strips for electrophoresis in a Spinco model R apparatus with 0.05 M Veronal buffer at pH 8.6 for 2 hours. After electrophoresis, the strips were dried and scanned for radioactivity with a Vanguard autostrip scanner. The behavior of saccharopine and lysine under these conditions has been described elsewhere (2). The areas of the peaks were approximated thus allowing an estimation of the percentage of conversion of lysine to saccharopine.

The enzyme assay most commonly employed was based on the decrease in optical density at 340 μm (1-cm light path) which occurred as NADH was oxidized. A Beckman model DU spectrophotometer was used for these measurements. Each cuvette contained 100 μmoles of l-lysine, 20 μmoles of 2-oxoglutarate, 250 μmoles of potassium phosphate buffer, pH 7.0, 0.25 μmole of NADH, and sufficient enzyme solution and water to make the final volume to 2.0 ml. The cuvette contents were mixed by inversion and readings were taken every 30 sec for 3 min. The blank contained 20 μmoles of oxoglutarate in water. One unit of enzyme is defined as that amount which catalyzes the oxidation of NADH at the rate of 0.01 optical density unit per min at 25°C. The reaction was linear with respect to time and enzyme concentration as shown in Fig. 1.

**Purification of Saccharopine Dehydrogenase**—The establishment of saccharopine dehydrogenase as a sulfhydryl enzyme, as described in a later section, prompted the addition of a reducing agent, 2-mercaptoethanol, and a chelator, EDTA, to all of the buffers used in conjunction with the enzyme. Glass-distilled water was used throughout the purification procedure with the exception of the initial dialysis step. All of the procedures were carried out below 5°C. Protein was determined by the method of Lowry et al. (7).

Fleischmann's bakers' yeast was obtained in 8 to 15 pound quantities and the cells were disrupted by freezing with liquid nitrogen followed by rapid thawing. The cell debris was removed immediately by centrifugation at 13,200 × g for 15 min. The crude extract obtained was dialyzed for 6 hours against 0.005 M standard buffer (standard buffer refers to the stated molarity of potassium phosphate buffer at pH 7.0, containing 10^-2 M 2-mercaptoethanol and 10^-4 M EDTA) and centrifuged at 13,200 × g for 20 min. The resulting extract (Fraction I) was assayed immediately for protein and diluted to a protein concentration of 13 mg per ml with 0.005 M standard buffer just prior to beginning the purification procedure. Fraction I was first fractionated by the dropwise addition of precooled absolute ethanol to a final concentration of 10% by volume after which the preparation was immediately cooled to -4°C in a Dry Ice-acetone bath. The solution was allowed to remain at this temperature with stirring for 15 min and then centrifuged in a Sorvall RC-2 refrigerated centrifuge at 13,200 × g for 20 min. The precipitate was then dissolved in a minimum amount of 0.1 M standard buffer. At this point the enzyme preparation could be left standing overnight at 5°C without appreciable loss of enzyme activity.

Prior to beginning fractionation with salt, the protein concentration was adjusted to 3.0 mg per ml with 0.1 M standard buffer (Fraction II). To Fraction II was added powdered ammonium sulfate to 50% saturation with stirring, and it was then allowed to remain in the cold with stirring for 30 min. The precipitate was obtained by centrifugation as described above and discarded. To the supernatant solution was added powdered ammonium sulfate to 70% saturation and the precipitate containing the enzyme was obtained as described, dissolved in 0.01 M standard buffer, and dialyzed for 4 hours against approximately 20 volumes of 0.005 M standard buffer (Fraction III). The next purification step was begun immediately in order to avoid large losses of enzymatic activity.

Alumina gel Cy, prewashed with glass-distilled water containing 10^-3 M 2-mercaptoethanol and 10^-4 M EDTA and resuspended in washing solution, was added to Fraction III to give a gel to protein ratio of 0.2. The suspension was stirred at 5°C for 20 min and the gel was harvested by centrifugation. The gel was then washed three times with 25-ml fractions of 0.1 M standard buffer, pH 7.0, and three times with 25-ml fractions of 0.25 M standard buffer, pH 7.5. The majority of the enzyme activity appeared in the first of the 0.25 M washings. The enzyme solution was dialyzed against 20 volumes of 0.005 M standard buffer for 4 hours. The dialyzed preparation, Fraction...
immediately combined and concentrated by reverse dialysis were collected. The enzyme was not retained by the column and eluted with the same buffer. Fractions of 5 to 10 ml each of cellulose, previously equilibrated with 0.005 M standard buffer, IV, was then applied to a column (2.5 X 10 cm) of DEAE-cellulose, previously equilibrated with 0.005 M standard buffer, containing 0.1 M KCl overnight against 0.01 M standard buffer containing 0.1 M KCl. The resulting solution was dialyzed overnight against 0.01 M standard buffer containing 0.1 M KCl (Fraction V). A typical purification scheme is shown in Table I. Fraction V representing a 700-fold purification was employed as the enzyme preparation in the following experiments.

| Fraction | Protein (mg) | Units | Specific activity | Purification | Recovery (%)
<table>
<thead>
<tr>
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<tbody>
<tr>
<td>I. Crude enzyme</td>
<td>5,625</td>
<td>88,500</td>
<td>10.4</td>
<td>-fold</td>
<td>100</td>
</tr>
<tr>
<td>II. Ethanol, 0 to 10%</td>
<td>735</td>
<td>44,800</td>
<td>61</td>
<td>1</td>
<td>5.8</td>
</tr>
<tr>
<td>III. (NH₄)₂SO₄, 5 to 70%</td>
<td>180</td>
<td>26,300</td>
<td>146</td>
<td>14</td>
<td>45</td>
</tr>
<tr>
<td>IV. Alumina gel C₅ eluate</td>
<td>9.2</td>
<td>10,700</td>
<td>1,163</td>
<td>112</td>
<td>18</td>
</tr>
<tr>
<td>V. DEAE-cellulose eluate</td>
<td>1.3</td>
<td>9,660</td>
<td>7,370</td>
<td>708</td>
<td>16</td>
</tr>
</tbody>
</table>

IV, was then applied to a column (2.5 X 10 cm) of DEAE-cellulose, previously equilibrated with 0.005 M standard buffer, and eluted with the same buffer. Fractions of 5 to 10 ml each were collected. The enzyme was not retained by the column and appeared between Fractions 5 and 18. These fractions were immediately combined and concentrated by reverse dialysis against Carboxax C-4000. The resulting solution was dialyzed overnight against 0.01 M standard buffer containing 0.1 M KCl (Fraction V). A typical purification scheme is shown in Table I. Fraction V representing a 700-fold purification was employed as the enzyme preparation in the following experiments.

Absorption Spectrum—The ultraviolet absorption spectrum of Fraction V revealed a maximum between the wave lengths at 275 and 280 m. A minor absorption was observed in the visible region at approximately 420 m. The relevance of this absorption, if any, to saccharopine dehydrogenase has not been determined.

RESULTS

Saccharopine-Lysine Relationship—Although preliminary attempts to form lysine from saccharopine in cell-free extracts had failed (6), it was found that reverse Reaction 2 could readily be carried out by such preparations. When a crude extract was passed through a Sephadex G-50 column as previously described (6), an enzyme fraction was obtained free from smaller molecules. When 40 mg of this fraction were incubated with lysine-14 C, 2-oxoglutarate, and NADH under the conditions previously described (cf. "Enzyme Assays"), a 42% conversion of lysine to saccharopine could be shown. Oxoglutarate and NADH were obligatory for saccharopine synthesis indicating that saccharopine is indeed being formed by the oxidative condensation of lysine with oxoglutarate. By altering the conditions of the enzymatic reaction, it was soon observed that forward Reaction 2, i.e. the oxidative cleavage of saccharopine, could be effected by raising the pH of the system.

pH Optima of Forward and Reverse Reactions—The effect of pH was investigated for both the forward and reverse reactions catalyzed by saccharopine dehydrogenase. Two distinct optima were revealed as shown in Fig. 2. Reaction A displays a clear maximum around neutrality, while Reaction B, the forward reaction, proceeds more efficiently at higher pH values, the optimum being in the neighborhood of pH 10 or higher. Since H⁺ also directly participates in Reaction 2, it is likely that the pH activity curves of Fig. 2 reflect both an effect of H⁺ on the reaction rate as well as an effect on the equilibrium of the reaction. In any event, these data explain the apparent irreversibility of the reaction observed earlier (8). The conversion of aminoadipic acid to saccharopine was effected at a neutral pH (6). The consequent accumulation of saccharopine and the failure of the latter to be cleaved to form lysine was the result of the relatively high hydrogen ion concentration which tended to shift the equilibrium of the terminal reaction toward saccharopine.

Stoichiometry—The observed stoichiometry of the reaction catalyzed by saccharopine dehydrogenase is shown in Table II. The data are consistent with the assumption that the formation of 1 mole of saccharopine requires the utilization of 1 mole each of lysine, oxoglutarate, and NADH.

Inhibition by Sulfhydryl Reagents—p-Hydroxymercuribenzoate, at a concentration of 5 x 10^-4 M, inhibited the enzyme completely, while iodoacetic acid, at higher concentrations, had no effect as shown in Table III. The p-hydroxymercuribenzoate inhibition could be reversed almost entirely by the addition of sulfhydryl compounds, 2-mercaptoethanol and GSH being equally effective. The establishment of saccharopine dehydrogenase as a sulfhydryl enzyme stimulated experiments designed to assign a function to a sulfhydryl group in the mechanism of action of the enzyme. Preliminary work suggests that NADH is capable of protecting the enzyme against sulfhydryl reagents.

![Image of a graph showing the pH optima of forward and reverse reactions.](http://www.jbc.org/)

**Table I**

| Fraction | Protein (mg) | Units | Specific activity | Purification | Recovery (%)
<table>
<thead>
<tr>
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<td>1.3</td>
<td>9,660</td>
<td>7,370</td>
<td>708</td>
<td>16</td>
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**Table II**

<table>
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<th>Reaction</th>
<th>Lysine</th>
<th>Oxoglutarate</th>
<th>NADH</th>
<th>Saccharopine</th>
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<tr>
<td>Before incubation</td>
<td>0.75</td>
<td>0.75</td>
<td>0.69</td>
<td>0</td>
</tr>
<tr>
<td>After incubation</td>
<td>0.55</td>
<td>0.53</td>
<td>0.48</td>
<td>0.18</td>
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<tr>
<td>Change</td>
<td>-0.20</td>
<td>-0.22</td>
<td>-0.21</td>
<td>+0.18</td>
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</table>
Saccharopine dehydrogenase inhibition by sulfhydryl reagents

Reactions were run as described for the spectrophotometric enzyme assay procedure. Inhibitors were preincubated with purified enzyme for 60 sec prior to the addition of substrates. The enzyme preparation used in these experiments had been previously dialyzed against 2-mercaptoethanol-free buffer. In the experiments concerned with effects of 2-mercaptoethanol and GSH, these reagents were added at the time of substrate addition.

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Additions to reaction mixture</th>
<th>Inhibition</th>
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<tbody>
<tr>
<td>p-Hydroxymercuribenzoate, 2.5 X 10^{-4} M</td>
<td>None</td>
<td>22</td>
</tr>
<tr>
<td>p-Hydroxymercuribenzoate, 5.0 X 10^{-3} M</td>
<td>None</td>
<td>100</td>
</tr>
<tr>
<td>p-Hydroxymercuribenzoate, 5.0 X 10^{-4} M</td>
<td>2-Mercaptoethanol, 5 X 10^{-3} M</td>
<td>4</td>
</tr>
<tr>
<td>p-Hydroxymercuribenzoate, 5.0 X 10^{-4} M</td>
<td>GSH, 5 X 10^{-3} M</td>
<td>13</td>
</tr>
<tr>
<td>Iodoacetate, 1.0 X 10^{-4} M</td>
<td>None</td>
<td>6</td>
</tr>
</tbody>
</table>

Table IV

Coenzyme specificity and analogue inhibition

In addition to the coenzymes, the reaction mixtures contained 10 μmoles of saccharopine and 100 μmoles of glycine-NaOH buffer at pH 9.5. In Experiment 1 the reaction mixtures contained 2 μmoles of coenzyme. In Experiment 2, 0.6 μmole of NAD was used.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Coenzyme</th>
<th>Inhibitor</th>
<th>Relative rate of reduction</th>
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<tr>
<td>1</td>
<td>NAD</td>
<td></td>
<td>100</td>
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<tr>
<td></td>
<td>NADP</td>
<td></td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>3-Acetylpyridine adenine dinucleotide</td>
<td></td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>3-Pyridinedehyde adenine dinucleotide</td>
<td>Nicotinamide hypoxanthine dinucleotide</td>
<td>41</td>
</tr>
<tr>
<td></td>
<td>3-Pyridinedehyde-nicotinamide hypoxanthine dinucleotide</td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>NAD</td>
<td>3-Acetylpyridine adenine dinucleotide, 1 X 10^{-3} M</td>
<td>96</td>
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<tr>
<td></td>
<td>NAD</td>
<td>3-Pyridinedehyde adenine dinucleotide, 1 X 10^{-3} M</td>
<td>81</td>
</tr>
<tr>
<td></td>
<td>NAD</td>
<td>3-Pyridinedehyde adenine dinucleotide, 1.5 X 10^{-3} M</td>
<td>66</td>
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</table>

Reaction mechanisms involving reduction and re-formation of a protein disulfide group have been implicated in a few cases at the enzymatic level (9, 10). Such a possibility was considered for saccharopine dehydrogenase, and accordingly, the system was tested for its sensitivity to arsenite inhibition. The presence of arsenite, along with a suitable reducing agent, had no apparent effect on the reaction rate. On the basis of this observation, the association of a disulfide with the active site of the enzyme is considered improbable.

Substrate Specificity—An absolute specificity was exhibited by the enzyme for L-lysine in the reverse of Reaction 2. At the levels tested (0.05 M), no activity was observed with the next lower homologue, ornithine, nor with α-N-acetylysine, ε-N-acetylysine, ε-N-methyllysine, or ammonium ion. A similar situation existed with respect to the α-keto acid specificity. When 0.01 M oxoglutarate was omitted, equimolar amounts of pyruvate and oxobutyrate showed only traces of activity, while oxalacetic acid and oxosodic acid supported no reaction at all.

Coenzyme Specificity—A marked preference was also observed toward the coenzyme (Table IV). NADPH was oxidized at a rate of only 5% that of NADH. When the reaction was reversed, NADP+ could not be substituted for NAD+. A comparison of the rates of reduction of a series of NAD+ analogues revealed that saccharopine dehydrogenase would accept only nicotinamide hypoxanthine dinucleotide. No reaction was observed with any of the other three analogues tested as shown in Table IV. The effectiveness of nicotinamide hypoxanthine dinucleotide and the poor acceptance of the other analogues tested are similar to observations on the yeast alcohol dehydrogenase system (11).

Although acetylpyridine adenine dinucleotide and pyridinedehyde adenine dinucleotide are not capable of supporting the reaction catalyzed by saccharopine dehydrogenase, they do appear capable of binding with the enzyme. This is suggested by the data shown in Table IV in which it is seen that the reduction of NAD+ can be inhibited to a slight extent by acetylpyridine adenine dinucleotide and more effectively by pyridinedehyde adenine dinucleotide.

Stability—Considerable difficulty was encountered in maintaining the activity of the enzyme throughout its purification and storage. This was overcome partially by the presence of 2-mercaptoethanol, 1 X 10^{-3} M. Another factor, even more critical, has proven to be the ionic strength of the medium. Enzymatic activity was markedly dependent upon the concentration of salt. At a protein concentration of 40 μg per ml, enzymatic activity dropped off sharply when the KCl concentration was below 0.1 M.

The pH stability of saccharopine dehydrogenase is shown in Fig. 3. The enzyme is fairly stable in the range of pH between 5 and 8. Alterations in pH to either extreme outside of these limits results in a marked loss of activity.

In the purified state, the enzyme is extremely sensitive to precipitation with ammonium sulfate, essentially all of the activity being lost. Consequently, it was necessary to resort to methods such as reverse dialysis against Carbowax for concentrating enzyme solutions.

Molecular Weight and Turnover Number—In order to obtain a rough estimate of the molecular weight of saccharopine dehydrogenase the enzyme was centrifuged in a sucrose gradient according to the method of Martin and Ames (12). Appropriate enzymes of known molecular weight were included as standards. The results of two such centrifuge runs are shown in Fig. 4. From these data an approximate molecular weight was calculated for saccharopine dehydrogenase and found to be 49,000. With the use of this number and data from Table I an approximate turnover number was calculated, 1,127 moles of NADH oxidized per min per mole of enzyme.

Michaelis Constants—The K_m values obtained for L-lysine,
Fig. 3. Stability of saccharopine dehydrogenase to pH. Aliquots of 1 ml each of saccharopine dehydrogenase, 40 μg of protein per ml, were dialyzed for 4 hours at 5°C against 100 ml of 0.05 M buffers, varying with respect to pH. Enzyme activity was measured spectrophotometrically. The buffers used were as described for Fig. 2.

EXPERIMENT 1

**YEAST ALCOHOL DEHYDROGENASE**

**SACCHAROPINE DEHYDROGENASE**

**ENZYME UNITS**

<table>
<thead>
<tr>
<th>DISTANCE FROM MENISCUS (cm)</th>
<th>25</th>
<th>19</th>
<th>13</th>
<th>7</th>
</tr>
</thead>
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<tr>
<td>0.084</td>
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<td>0</td>
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<td>1.68</td>
<td>6</td>
<td>4</td>
<td>2</td>
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</tr>
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<td>2.52</td>
<td>4</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

**FRACTION NUMBER**

Fig. 4. Sucrose density gradient centrifugation patterns of saccharopine dehydrogenase. Detectable amounts of yeast alcohol dehydrogenase (mol wt 150,000 (13)) and catalase (mol wt 248,000 (14)) were added to solutions of saccharopine dehydrogenase containing 0.8 μg per ml of purified saccharopine dehydrogenase. Aliquots of this solution, 0.1 ml, were layered onto sucrose gradients prepared as described by Martin and Ames (12) and containing 0.01 M standard buffer. The gradients were centrifuged at 39,000 rpm in a Spinco model L preparative ultracentrifuge with an SW-39 rotor for 12 hours and 20 min at 1°C. After centrifugation, the tubes containing the gradients were immediately punched and fractions of 11 drops each, 17.4 μl per drop, were collected. Spectrophotometric assays were employed for the detection of saccharopine dehydrogenase, alcohol dehydrogenase, and catalase.

DISCUSSION

From the data presented here, together with recent studies in our laboratory with yeast and Neurospora lysine auxotrophs (2, 3), it is clear that the reaction catalyzed by saccharopine dehydrogenase does indeed represent the terminal event of lysine biosynthesis in these organisms. Early doubts stemming from the apparent inability of cell extracts to effect the cleavage of saccharopine to form lysine (6) now seem to be entirely reasonable in view of the high pH required to effect their cleavage in a system in vitro (Fig. 2).

The high substrate specificity of saccharopine dehydrogenase, coupled with its ease of detection as described herein, has led to related studies to be published that indicate the presence of this enzyme may be regarded as diagnostic for the aminoadipic acid pathway (15, 16). In addition to yeast and N. crassa (2), the enzyme has been detected in certain species of lower and higher Ascomycetes as well as in Euglena gracilis. Diaminopimelic decarboxylase, the terminal enzyme of the diaminopimelic 2-oxoglutarate, and NADH were 1.2 × 10⁻⁴ M, 4.4 × 10⁻⁴ M, and 4.6 × 10⁻⁵ M, respectively. The high value for lysine may reflect the general function of the enzyme in the cell; that is, the enzyme exists to enable the organism to synthesize lysine. A high Kₘ value would tend to discourage the reverse reaction, thus allowing the cell to accumulate lysine in relatively high concentrations.
pathway of lysine biosynthesis, was notably absent in these instances. Such studies (15, 16) are in accord with Vogel's views of the phylogenetic distribution and dichotomy of lysine biosynthesis in lower plants (17).

A recent report of Higashino, Tsukada, and Lieberman (18) suggests that saccharopine may be involved in lysine catabolism in mammalian systems. Preparations of rat liver mitochondria were shown to degrade uniformly labeled 14C-lysine to 14C02. It was suggested that saccharopine may be involved in lysine catabolism (3), thus questioning the role of piperolic acid or related cyclic lysine metabolites in this process.

In considering in more detail the mechanism of amination of aminoadipic semialdehyde by glutamate to give lysine.

enzyme and substrate is also worthy of serious consideration. Studies on the mechanism of the reaction have been severely hampered by the liability of the enzyme and also by the difficulty of obtaining the protein in reasonably large quantities to permit studies of enzyme-substrate interaction.

The action of saccharopine dehydrogenase is not entirely unique, since octopine, α-N-(propionyl-2)-L-arginine, is formed by octopine dehydrogenase in a completely analogous manner (20).

\[ \text{Arginine} + \text{pyruvate} + \text{NADH} + \text{H}^+ \rightarrow \text{octopine} + \text{NAD}^+ + \text{H}_2\text{O} \]

It would also be interesting if lysopine, α-N-(propionyl-2)-L-lysine, an amino acid recently isolated from the crown gall bacillus (21), is synthesized from lysine, pyruvate, and reduced pyridine nucleotide. On the other hand, α-N-methyllysine which was not attacked by saccharopine dehydrogenase is cleaved oxidatively by α-alkyllysines, an enzyme present in animal tissues (22), to give lysine and formaldehyde. Also in the case of argininosuccinate, an amino acid that bears an N- succinoyl moiety, the mechanism of cleavage by argininosuccinase to give arginine and fumarate is entirely different from that exhibited by saccharopine dehydrogenase.

REFERENCES

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