Purification and Properties of an α-Ketoglutarate Transaminase from Escherichia coli*

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Purine can be converted to γ-aminobutyraldehyde by enzymes from different sources (1-3). It is generally believed that this conversion is catalyzed by a nonspecific diamine oxidase. The presence of such an enzyme in bacteria, however, has not been firmly established experimentally. We have previously reported that, in a mutant of Escherichia coli B selected to grow on putrescine as the sole source of carbon and nitrogen, the conversion of putrescine to γ-aminobutyraldehyde was catalyzed by a transaminase (4). In this paper, the purification and the properties of this enzyme are reported. The isolation and the characteristics of this mutant strain of Escherichia coli are reported elsewhere (5).

EXPERIMENTAL PROCEDURE

Putrescine-1,4-14C-dihydrochloride was purchased from New England Nuclear Corporation. Nonradioactive putrescine, other amines tested for substrate specificity, and o-aminobenzaldehyde were purchased from K and K Laboratories. L-Glutamic acid dehydrogenase was purchased from Nutritional Biochemicals Corporation.

Bacteria—The mutant of E. coli B was grown with aeration at room temperature in the following medium: Na2HPO4, 16.4 g; KH2PO4, 1.5 g; MgSO4, 0.97 g; CaCl2, 0.01 g; FeSO4, 0.01 g; and putrescine, 2 g (neutralized with HCl) per liter of deionized water. Cells were harvested at or near the end of log phase growth.

Enzymatic Assay—Putrescine α-ketoglutarate transaminase was assayed by an adaptation of the colorimetric method of Holmstedt, Larsson, and Tham (6). The standard incubation mixture contained in a total volume of 0.8 ml: 100 μmoles of α-ketoglutarate; 0.1 μmole of pyridoxal phosphate; 2 μmoles, of o-aminobenzaldehyde; and 0.1 ml, of enzyme preparation. The reaction was stopped at the appropriate time by the addition of 0.2 ml of 10% trichloroacetic acid, and the absorption at 435 μm was measured after 20 minutes. One unit of enzyme is defined as the amount of protein which converts 1 μmole of putrescine per minute to γ-aminobutyraldehyde under the standard conditions. The specific activity is the units of activity per mg of protein.

Protein was determined by the method of Warburg and Christian (7). Glutamic acid was qualitatively identified by paper chromatography (8) and quantitatively determined, after separation from excess of α-ketoglutarate by Dowex 50W-X4-H+ column, by the ninhydrin method (9), and by L-glutamic acid dehydrogenase. A small aliquot of eluent containing glutamic acid was dried over KOH under reduced pressure, and used for the substrate of L-glutamic acid dehydrogenase. The reaction mixture contained in a total volume of 1.0 ml: 500 μg, of L-glutamic acid dehydrogenase; 5 μmoles, of DPN+; and 100 μmoles, of sodium phosphate, pH 7.6. The reaction was stopped after 30 minutes of incubation at room temperature by the addition of 0.2 ml of 10% trichloroacetic acid. The absorption at 340 μm was measured, and the amount of L-glutamic acid was calculated from a standard curve of L-glutamic acid treated in the same way as described previously. α-Ketoglutarate was measured by the method of Friedemann (10).

For stoichiometry studies, unreacted α-ketoglutarate was isolated from the reaction mixture by passing through a Dowex 50W-X4-H+ column (0.5 × 10 cm). An additional 5 ml of deionized water were used to wash through all the α-ketoglutarate from the column. These two fractions were combined for α-ketoglutarate determination. The glutamate formed was then eluted from the column with 5 ml of 0.6 N HCl. An aliquot of this eluent was evaporated to dryness for L-glutamate determination. γ-Aminobutyraldehyde was identified by comparing the absorption spectrum of its coupling product with o-aminobenzaldehyde with that of synthetic Δ'-pyrroline (the cyclic form of γ-aminobutyraldehyde). Spectra from a representative experiment are shown in Fig. 1. Residual putrescine was eluted from the column with 10 ml of 3 N HCl. An aliquot was treated with 2,4-dinitrophenylhydrazine (100 μg/100 ml of 2 N HCl) for 30 minutes and then with Nort for 10 minutes. After the Nort was removed, the radioactivity of the supernatant was measured for the determination of the remaining putrescine.

RESULTS

Purification of Enzyme

Washed bacteria, 44 g, was suspended in 150 ml of 0.1 M Tris-HCl buffer, pH 7.3, with 100 μmoles of GSH and treated for 20 minutes in a Raytheon 10-ke sonic oscillator. Cell debris were removed by centrifugation at 10,000 × g for 20 minutes. The supernatant was then treated with 0.4 mg of DNase for 20 minutes at room temperature and centrifuged at 105,000 × g for 1 hour. The clear solution thus obtained had a volume of 153 ml and contained 37 mg of protein per ml. This was then rapidly heated to 50° and maintained between 50 and 53° for 5 minutes in a 55° water bath. The preparation was cooled rapidly to 0° in an ice bath and the brownish precipitate removed by centrifuging at 10,000 × g for 15 minutes. The supernatant was adjusted to pH 5.0 with cold 0.2 M acetic acid with constant
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FIG. 1. Absorption spectrum of 2,3-trimethylene-1,2-dihydroquinazolinium. I, reaction product; II, authentic pyrroline (γ-aminobutyraldehyde).

Fig. 2. Elution of the enzyme from DEAE-cellulose. Chromatography was performed as described in the text, and 0.1 ml of each fraction was assayed for the transaminase activity as described in the text.

stirring. After 20 minutes, the turbid solution was centrifuged at 10,000 × g for 15 minutes and the precipitate was redissolved in 150 ml of 0.1 M Tris-HCl buffer, pH 7.3. Successive portions of solid ammonium sulfate were added at 0-4° to reach 35, 40, 45, 50, 55, 60, 65, 70, and 75% saturation. The protein precipitated at each concentration of ammonium sulfate was collected by centrifuging for 10 minutes at 10,000 × g, dissolved in 5 to 10 ml of 0.1 M Tris-HCl buffer, pH 7.3, and dialyzed overnight against 4 liters of 0.001 M Tris-HCl buffer, pH 7.3. Most of the activity was found to precipitate between 50 and 65% saturation with ammonium sulfate. These fractions were, therefore, pooled, resulting in 32 ml of solution with 13.26 mg of protein per ml. To 31 ml of this solution was then added acetone, chilled to -15°, to reach 60% acetone by volume. The precipitate was quickly collected by centrifugation for 15 minutes at 10,000 × g, and dissolved in 11 ml of 0.03 M Tris-HCl buffer, pH 7.3. Of this solution, 10 ml were applied to a column of DEAE-cellulose1 (2 × 24 cm), previously equilibrated with 0.03 M Tris-HCl buffer, pH 7.3. The column was then subjected to gradient elution (11) with 300 ml of 0.03 M Tris-HCl buffer, pH 7.3, in the mixing flask and 500 ml of 0.5 M NaCl in the reservoir. The flow rate was approximately 1.5 ml per minute. The distribution of protein and enzyme in the fractions (3 ml each) is shown in Fig. 2. Fractions 65 to 88 containing 89% of recovered activity were pooled to give an 80-fold purified enzyme preparation at 33% yield. A summary of the purification procedure is given in Table I.

Stoichiometry and Identification of Products

α-Ketoglutamate, putrescine-1,4-14C, and a-aminobenzaldehyde, 2 μmoles each, were incubated with 14 μg of enzyme for 5 and 10 minutes at pH 9. These results are summarized in Table II. There was quantitative correlation between the amounts of products formed and the reactants utilized.

Properties

pH Optimum—The enzyme exhibited a sharp pH optimum, between 9 and 10, and was almost completely inactive at pH 7 (Fig. 3).

Kinetic Properties—The transaminase activity was a linear function of both incubation time and enzyme concentration (Fig. 4).

α-Ketocid Specificity—α-Ketoglutaric acid, pyruvic acid, and oxaloacetic acid were tested as possible substrates for this enzyme. Both α-ketoglutarate and pyruvate were active, with $K_m$ values of $8.8 \times 10^{-4}$ and $2.7 \times 10^{-4}$, respectively. Oxaloacetic acid was inactive. This differs from the behavior of the crude extract reported previously (4) where oxaloacetic acid could partially replace α-ketoglutaric acid. This is most likely caused by the decarboxylation of oxaloacetic acid to pyruvic acid by the crude extract. Since oxaloacetic acid decarboxylase is of wide occurrence in nature (12), its presence in the crude extract would be expected.

Table I

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Volume</th>
<th>Total activity</th>
<th>Total protein</th>
<th>Specific activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Soluble extract</td>
<td>150</td>
<td>135</td>
<td>5700</td>
<td>0.023</td>
</tr>
<tr>
<td>2. Heat treatment</td>
<td>138</td>
<td>110.4</td>
<td>4692</td>
<td>0.023</td>
</tr>
<tr>
<td>3. pH 5 treatment</td>
<td>150</td>
<td>105</td>
<td>1877</td>
<td>0.075</td>
</tr>
<tr>
<td>4. Ammonium sulfate</td>
<td>32</td>
<td>54.4</td>
<td>437.4</td>
<td>0.124</td>
</tr>
<tr>
<td>5. Acetone treatment</td>
<td>30</td>
<td>48</td>
<td>360.0</td>
<td>0.133</td>
</tr>
<tr>
<td>6. DEAE-cellulose eluates</td>
<td>42</td>
<td>37.8</td>
<td>23.1</td>
<td>1.63</td>
</tr>
</tbody>
</table>

Table II

Stoichiometry of reaction

Experiments 1 and 2 were incubated for 5 and 10 minutes, respectively. After incubation, the reactions were stopped by acidification of the reaction mixture by the addition of Dowex 50W-X4 resin.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Reactants</th>
<th>Products</th>
</tr>
</thead>
<tbody>
<tr>
<td>Putrescine</td>
<td>α-Ketoglutarate</td>
<td>l-Glutamate γ-Aminobutyraldehyde</td>
</tr>
<tr>
<td>1</td>
<td>-0.48</td>
<td>-0.48</td>
</tr>
<tr>
<td>2</td>
<td>-0.82</td>
<td>-0.84</td>
</tr>
</tbody>
</table>

1 DEAE-cellulose, diethylaminoethyl-cellulose.
**Amine Specificity**—Several amines and amino acids were tested as substrate with α-ketoglutarate as cosubstrate. The results are shown in Table III. Since the method of Holmstedt does not apply to most of the amines tested, the reactions were assayed by isolation and determination of the L-glutamate formed. Activity was observed with three diamines; 1,4-diaminobutane (putrescine), 1,5-diaminopentane (cadaverine), and 1,7-diaminoheptane. The first two were equally active and much more active than the last. 1,6-Diaminohexane was not tested, but would be expected to be active. 1,3-Diaminopropane was completely inactive, as were lysine, ornithine, spermidine, and several basic amino acids which can serve as substrate for diamine oxidase. The enzyme also contained transaminase activity toward γ-aminobutyrate. However, this was much weaker in the purified preparation than in the crude extract and was undoubtedly caused by contamination by γ-aminobutyrate-α-ketoglutarate transaminase, which was present in the putrescine-grown cells, and was necessary for the conversion of putrescine to succinate.

**Pyridoxal Phosphate Requirement**

In a preliminary communication (4), we reported the requirement of pyridoxal phosphate by a partially purified enzyme preparation. Subsequent work showed that the dependence on pyridoxal phosphate varied from one enzyme preparation to another. Although some preparations showed a 10-fold stimulation by the addition of pyridoxal phosphate, others showed slight or no stimulation. This variation is illustrated later in Tables IV and V. Since the cause for this variation could not be ascertained, other methods, ultraviolet irradiation (13, 14) and phenylhydrazine treatment, were used to show the requirement for pyridoxal phosphate.

The ultraviolet irradiation was performed at 4° with a Mineralite model No. SL 2537 shortwave ultraviolet scanning lamp from Ultra-Violet Products, Inc., South Pasadena, California, at a

![Fig. 3. Enzyme activity as a function of pH. Sodium phosphate buffer, 0.1 M, was used for pH 7 and 7.5. For the higher pH values, 0.1 M Tris-HCl buffers were used. Other reaction conditions were the same as the standard conditions. The reactions were carried out at room temperature for 30 minutes.](image)

![Fig. 4. Linearity of the diamine α-ketoglutarate transaminase reaction. Curve a shows the effects of increasing concentration of enzyme; the incubation time was 10 minutes. Curve b shows the absorbance change as a function of time. The amount of enzyme used was 0.17 mg of protein. Other assay conditions were the same as those described in the text.](image)

![TABLE III Substrate specificity](image)

Of each substrate, 5 μmoles were used in the standard reaction mixture. After 1 hour of incubation, the reaction was stopped by boiling, the glutamate formed was isolated by the use of a Dowex 50W-X4 column (0.6 x 7 cm) and assayed as described in the text. The enzyme used was a partially purified preparation.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1,3-Diaminopropane</td>
<td>0</td>
</tr>
<tr>
<td>1,4-Diaminobutane (putrescine)</td>
<td>100</td>
</tr>
<tr>
<td>1,5-Diaminopentane (cadaverine)</td>
<td>107</td>
</tr>
<tr>
<td>1,7-Diaminoheptane</td>
<td>30</td>
</tr>
<tr>
<td>Histamine</td>
<td>0</td>
</tr>
<tr>
<td>Tyramine</td>
<td>0</td>
</tr>
<tr>
<td>Spermidine</td>
<td>0</td>
</tr>
<tr>
<td>γ-Aminobutyric acid</td>
<td>14</td>
</tr>
<tr>
<td>4-Aminobutanol</td>
<td>0</td>
</tr>
<tr>
<td>β-Alanine</td>
<td>0</td>
</tr>
<tr>
<td>DL-Ornithine</td>
<td>1</td>
</tr>
<tr>
<td>L-Lysine</td>
<td>0</td>
</tr>
</tbody>
</table>

* The + represents the specific activity in the presence of 0.1 μmole of pyridoxal phosphate and the − represents the activity in the absence of added pyridoxal phosphate. Other assay conditions were the same as those described in the text.

![TABLE IV Effect of ultraviolet irradiation](image)

![TABLE V Effect of phenylhydrazine treatment](image)

* Refer to Table IV.
distance of 10 cm. The depth of the solution was 0.5 cm. The results of these experiments are shown in Table IV. It can be seen that 10 hours of irradiation resulted in almost absolute requirement of pyridoxal phosphate with little loss in enzyme activity. With 36 hours of irradiation, there was substantial inactivation of the enzyme.

For the phenylhydrazine treatment, the protein in 1 ml of enzyme solution was precipitated by the addition of solid ammonium sulfate at pH 8.8 to 75% saturation. To this protein suspension, 0.2 ml of 10⁻² M phenylhydrazine hydrochloride in methanol was added and maintained at 0° for 20 minutes. The protein was collected by centrifugation and dissolved in 1 ml of 0.1 M Tris-HCl buffer, pH 9. This solution was then treated with a small amount of Norit at room temperature for 10 minutes. After removal of Norit by centrifugation, the solution was dialyzed twice against 3 liters of 0.001 M Tris-HCl buffer, pH 8.8, for a total of 12 hours. The results of these treatments, which are shown in Table V, clearly illustrate the requirement of pyridoxal phosphate for the activity.

**DISCUSSION**

Two different types of enzymatic oxidation of putrescine to $\gamma$-aminobutyraldehyde have been reported. Diamine oxidase, which has been highly purified from beef plasma by Tabor (2), is known to catalyze the following reaction,

$$\text{RCH}_2\text{NH}_2 + \text{O}_2 + \text{H}_2\text{O} \rightarrow \text{RCHO} + \text{NH}_3 + \text{H}_2\text{O}_2$$

Another enzyme, putrescine dehydrogenase from Achromobacter species was reported to catalyze the oxidation of putrescine by oxygen in the presence of the respiratory system (10). However, this enzyme has not been well characterized.

Neither diamine oxidase nor putrescine dehydrogenase can be detected in dialyzed crude extracts from E. coli B adapted to grow on putrescine as the sole source of nitrogen or from the mutant studied here. Attempts to detect ammonia formation from the dialyzed crude extracts with Nessler's reagent were unsuccessful and it was concluded that these enzymes were absent in the extract.

The transaminase described here has two features which are different from other transaminases. First, the pH optimum for this enzyme is between 9 and 10, at which pH some of the pyridoxal phosphate containing enzymes are inactivated owing to the dissociation of the pyridoxal phosphate (15). Second, the substrate is a diamine with no carboxyl group. Although transamination involving amino groups at positions $\alpha$, $\gamma$, or $\delta$ from carboxyl group have been reported, this appears to be the first transaminase utilizing a substrate with no carboxyl group.

**SUMMARY**

A diamine $\alpha$-ketoglutaric acid transaminase has been purified about 80-fold from the extract of an *Escherichia coli* mutant. The enzyme has an unusually high pH optimum (between 9 and 10). The substrates for the enzyme are terminal diamines with 4 to 7 carbon atoms and $\alpha$-ketoglutarate or pyruvate. The pyridoxal phosphate requirement for enzymatic activity was shown by the nonspecific effects of ultraviolet irradiation or of treatment with phenylhydrazine in the presence or absence of added pyridoxal phosphate.

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**REFERENCES**

7. Warrburg, O., and Christian, W., Biochem. Z., 310, 384 (1941).
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