Branched Chain Amino Acid Aminotransferase of Salmonella typhimurium

II. KINETIC COMPARISON WITH THE ENZYME FROM SALMONELLA MONTEVIDEO*

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SUMMARY

The branched chain amino acid aminotransferases (transaminase B) of Salmonella typhimurium and Salmonella montevideo have been purified and studied kinetically. The results of partial kinetic characterization of the enzyme support the conclusion that the reaction proceeds via a binary "ping-pong" mechanism. The Michaelis constants for the amino acceptor, α-ketoglutarate, and three branched chain amino acid donors (isoleucine, leucine, and valine) were determined with the use of an appropriate Fortran computer program. No significant kinetic differences were found between the enzymes of the two Salmonella species. The $K_m$ for α-ketoglutarate is the same with each of the branched chain amino acids. The aminotransferase displays a comparable affinity for isoleucine and leucine, which is greater than that observed for valine.

EXPERIMENTAL PROCEDURE

Materials—Commercial products utilized in the assays include α-ketoglutarate, L-isoleucine, L-valine, and L-leucine, purchased from General Biochemicals; and pyridoxal phosphate (Nutritional Biochemicals Corporation). Collodion bags were obtained from Carl Schleicher and Schuell Company.

Purification Procedures—The procedure used to purify the enzyme has been described in detail by Coleman and Armstrong (2). The protocol involves the use of Salmonella cells grown under derepressed conditions to prepare cell-free extracts. Such extracts routinely possess 4- to 7-fold transaminase activity observed in extracts of wild-type Salmonella. The extracts were subjected to: (a) heat treatment; (b) ammonium sulfate fractionation; and (c) DEAE-cellulose chromatography to obtain the purified preparations. The purified enzyme was then concentrated in collodion bags under reduced pressure to a volume containing approximately 20 mg per ml. Such concentrates can be stored in the cold without loss of activity for a period of several weeks. Specific activities, defined as micromoles of keto acid product formed per mg protein per hour, in the range of 2000 to 2500 were obtained for both the S. typhimurium and S. montevideo enzymes. The S. typhimurium transaminase can be crystallized from such a purified preparation (2); however, the S. montevideo activity cannot. Thus, to ensure the use of comparable enzyme preparations in the study, the purified preparations of the enzymes from both species were used. Based on the specific activity of the crystalline enzyme of S. typhimurium (2), which contains no significant contaminants, the enzymes used in this study are 45 to 55% pure.

Assay—Details of the assay used have been reported by Coleman and Armstrong (2). The assay involves the selective formation and extraction of the 2,4-dinitrophenylhydrazine derivative of the α-keto acid product. Thus, the behavior of both the α-keto acid substrate and product in the system determines the usefulness of the assay. The assay has proved to be
of value in studies that use α-ketoglutarate as amino acceptor and the branched chain amino acids (isoleucine, valine, and leucine) as amino donors. It is in this fashion that the assays have been carried out in this study, i.e. the reverse of the bio-

**TABLE I**

**Purification of branched chain amino acid aminotransferase of S. typhimurium and S. montevideo**

For the enzymatic data presented, α-ketoglutarate served as the amino acceptor and L-isoleucine as the amino donor.

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Specific Activity</th>
<th>Over-all Recovery</th>
<th>Over-all Purification</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. typhimurium</td>
<td>S. montevideo</td>
<td>S. typhimurium</td>
<td>S. montevideo</td>
</tr>
<tr>
<td>Protein mg</td>
<td>%</td>
<td>%</td>
<td>-fold</td>
</tr>
<tr>
<td>Cell-free extract</td>
<td>31</td>
<td>34</td>
<td>100</td>
</tr>
<tr>
<td>Heat treatment</td>
<td>173</td>
<td>195</td>
<td>82</td>
</tr>
<tr>
<td>(NH₄)₂SO₄</td>
<td>730</td>
<td>768</td>
<td>80</td>
</tr>
<tr>
<td>DEAE-cellulose</td>
<td>2228</td>
<td>2063</td>
<td>56</td>
</tr>
</tbody>
</table>

**Analysis of Data**—The data were initially analyzed graphically by plotting reciprocal velocities against reciprocal substrate concentrations. Intercepts obtained from the primary plots were then plotted against reciprocals of the cosubstrate concentrations. Because a series of parallel lines could be derived from the primary plots, a binary ping-pong mechanism was indicated (3). A Fortran computer program, kindly provided by Professor W. W. Cleland of the University of Wisconsin, was then used for the analysis of the initial velocity kinetics of transamination. This program fits data directly to the following equation.

\[ v = \frac{V_{\text{AB}}}{K_B + K_A + AB} \]

Equal variance for the velocities (expressed in specific activities) is assumed. The terms in the equation are: \( v \) = observed velocity; \( V \) = maximum velocity; \( A \) and \( B \) = concentrations of reactants; and \( K_A \) and \( K_B \) = Michaelis constants of the reactants.

**Fig. 1.** Initial velocity pattern for the enzyme from *S. typhimurium* with α-ketoglutarate as varied substrate. Velocity units are micromoles of keto acid formed per mg of protein per hour. L-Isoleucine concentrations: 1, 2.0 mM; 2, 1.5 mM; 3, 1.0 mM; 4, 0.75 mM; and 5, 0.5 mM. Enzyme concentration: 9 × 10⁻⁶ mg per ml.

**Fig. 2.** Initial velocity pattern for the enzyme from *S. typhimurium* with L-isoleucine as varied substrate. Velocity units are micromoles of keto acid formed per mg of protein per hour. α-Ketoglutarate concentrations: 1, 5 mM; 2, 4 mM; 3, 3 mM; and 4, 2 mM. Enzyme concentration: 9 × 10⁻⁶ mg per ml. The inset is a secondary plot of the intercepts of the lines versus the reciprocals of the fixed substrate concentrations. \( A \), intercepts versus reciprocal of L-isoleucine concentrations from Fig. 1. \( B \), intercepts versus reciprocal of α-ketoglutarate concentrations from Fig. 2.

**TABLE II**

**Kinetic constants determined with use of Fortran program for α-ketoglutarate and branched chain amino acids**

<table>
<thead>
<tr>
<th>Species</th>
<th>( K_m ) values for amino acceptor</th>
<th>( K_m ) values for amino donors</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>α-Ketoglutarate</td>
<td>Isoleucine</td>
</tr>
<tr>
<td></td>
<td>Donor: isoleucine</td>
<td>Donor: leucine</td>
</tr>
<tr>
<td><em>S. typhimurium</em></td>
<td>3.0 ± 0.3</td>
<td>3.4 ± 0.3</td>
</tr>
<tr>
<td><em>S. montevideo</em></td>
<td>3.7 ± 0.5</td>
<td>3.6 ± 0.4</td>
</tr>
</tbody>
</table>
The kinetic data were processed on an IBM 1130 digital computer.

RESULTS

Enzyme Purification—A summary of the results of the purification of the branched chain amino acid aminotransferase of S. typhimurium and S. montevidae is presented in Table I. As seen, the initial activities observed in the cell-free extracts of both species are essentially the same, and the extent of purification per step is very comparable. Thus, in the purification procedures, no differences were noted that were greater than those routinely expected in the preparation of separate cell-free extracts from the same source. It should be noted that because these extracts were prepared from mutant cells grown under derepressed conditions (2), the final overall purification (60- to 70-fold) is equivalent to a purification of 300- to 350-fold of wild-type Salmonella activity.

Kinetic Data—For both the S. typhimurium and the S. montevidae enzymes, sets of assays were carried out in which a-ketoglutarate served as the amino acceptor and isoleucine, valine, or leucine as the amino donor. Figs. 1 and 2 show the family of lines derived for the S. typhimurium enzyme with L-isoleucine as amino donor and a-ketoglutarate as acceptor. In Fig. 1, isoleucine and a-ketoglutarate are the fixed and varied substrates, respectively; the same data with a-ketoglutarate as the fixed substrate and isoleucine as the varied substrate are presented in Fig. 2. The inset in Fig. 2 is the secondary plot of intercepts versus reciprocal concentrations of fixed substrate. These plots are linear and intercept at 1/Vmax.1

Computer Analyses of Data—To determine the Michaelis constants for the various reactants employed in this study, the kinetic data were processed through a Fortran computer program that was designed for the analysis of enzymatic reactions that involve a binary ping-pong mechanism (5). Results of these analyses are presented in Table II. The Kₘ values obtained for a-ketoglutarate, whether with the S. typhimurium or S. montevidae enzyme or any of the three amino donors, are very similar (an average value of 3.3 mm). The Kₘ values for each of the amino donors with either of the two enzymes are also very comparable. L-isoleucine and L-leucine have values that are very close to each other, in the range of 1.1 to 1.6 and 2.0 to 2.4 mm, respectively. Both enzymes display less affinity for L-valine (Kₘ = 5.1 to 5.5 mm) than for the other two amino donors.

DISCUSSION

This study on the branched chain amino acid aminotransferase of two species of Salmonella was primarily concerned with a preliminary kinetic characterization of the enzyme. By varying the concentrations of both amino acceptor and donor in a series of assays, data that are representative of a binary ping-pong mechanism (3) were collected on the enzyme from the two Salmonella species. To obtain a meaningful evaluation of these data, they were analyzed with the use of a Fortran computer program (3, 5). The Kₘ values obtained for the amino acceptor (a-ketoglutarate) and three amino donors (isoleucine, leucine, and valine) are very comparable for the two Salmonella species (Table II). Because of the close relatedness of these two bacterial species, these results are not surprising. With both enzymes, the Kₘ values for a-ketoglutarate with the three amino donors are essentially the same. When the standard deviations recorded in Table II are considered, the Kₘ value for a-ketoglutarate with the S. typhimurium enzyme is 2.7 to 3.7 mm; and with the S. montevidae enzyme, it is 2.3 to 1.2 mm. In the case of the amino donors, isoleucine and leucine possess very similar Kₘ values, i.e. 0.7 to 2.0 mm for isoleucine and 1.7 to 2.6 mm for leucine. Each of the two Salmonella enzymes shows an affinity for valine (4.7 to 6.0 mm) that is less than those observed for the other two amino acids.

Michaelis constants for a-ketoglutarate and the three branched chain amino acids have been determined for the supernatant and mitochondrial forms of the branched chain amino acid aminotransferase of hog heart (6). These Kₘ values were obtained by varying the level of one substrate at a fixed level of the other substrates. Because of the binary ping-pong mechanism involved in the reaction, such determinations would yield apparent Michaelis constants and are, therefore, of limited value for comparison with our data. The supernatant enzyme from hog heart has the following kinetic constants: 11.0 mm for valine; 3.8 mm for both isoleucine and leucine; and 0.6 mm for a-ketoglutarate. These results are, for the most part, similar to those obtained with the Salmonella enzymes in that the Kₘ values for isoleucine and leucine are lower than those for valine. The exception is that, whereas the microbial enzyme shows a greater affinity for isoleucine and leucine than for a-ketoglutarate, the hog heart enzyme shows its greatest affinity for a-ketoglutarate. The Kₘ values for the mitochondrial enzyme from hog heart are: 1.3 mm for valine; 0.4 mm for isoleucine and leucine; and 2.2 mm for a-ketoglutarate. The Kₘ values for isoleucine and leucine and for a-ketoglutarate are essentially reversed in these two forms of the enzyme of hog heart. However, data on the three enzymes are consistent in the observation that the values for isoleucine and leucine are less (approximately 3-fold) than that for valine.

This initial kinetic study on the microbial aminotransferase is limited in scope because of the limitations of the assay utilized. Attempts are currently underway to develop an assay that will possess the versatility required for a thorough examination of the kinetics of the reaction. Nevertheless, the data obtained and subsequently subjected to computer analysis have provided meaningful basic kinetic information about the branched chain amino acid aminotransferase of Salmonella.

REFERENCES
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