**d-Amino Acid Aminotransferase of Bacillus sphaericus**

**ENZYMOLYC AND SPECTROMETRIC PROPERTIES**

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The first evidence for the occurrence of d-amino acid specific aminotransferase was found by Thorne et al. (1, 2) in the cell-free extract of *Bacillus subtilis* and *Bacillus anthracis*. They showed that the enzyme of *B. subtilis* was activated a few-fold by pyridoxal-5'-P and suggested that this compound was a cofactor. d-Amino acid aminotransferase was subsequently demonstrated in extracts of *Rhodospirillum rubrum* (3), *Bacillus sphaericus* (4), and *Bacillus licheniformis* (5), and recently has also been found in higher plants (6, 7) and mammalian liver (8). d-Alanine aminotransferase was purified to near homogeneity from *B. subtilis* to elucidate the enzymologic and kinetic properties (9–11). Exact knowledge of the enzymatic transamination of d-amino acids is of value not only for a study of the enzyme chemistry of d-amino acid metabolism, but also for understanding the reaction mechanisms of L-amino acid aminotransferases and amino acid racemases. We found a high activity of d-amino acid aminotransferase in the cell-free extract of *B. sphaericus* IFO 3525 (12), and recently have purified the enzyme to homogeneity and crystallized it (13). In the present paper, more detailed studies on enzymologic and physicochemical characteristics of the crystalline d-amino acid aminotransferase are described with particular emphasis on the bound pyridoxal-5'-P.

**MATERIALS AND METHODS**

**Chemicals**—d-Cycloserine was obtained from Shionogi Seiyaku Co., Osaka; d-, l-, and o- penicillamine from Calbiochem; Sephadex G-25 from Pharmacia, Upsala, Sweden; Bio-Gel P-150 from Bio-Rad Laboratories, Richmond, California; pyridoxal-5'-P and d-amino acids from Kyowa Hakko Kogyo, Tokyo; and aminoxyacetate, o-keto acids, pyridoxal, pyridoxamine, pyridoxamine-5'-P, and pyridoxine-5'-P from Nakarai Chemicals, Kyoto, Japan. d-Amino acids were products of Ajinomoto Co., Tokyo. Pyridoxamine-5'-P and pyridoxal-5'-P were chromatographically purified by the method of Peterson and Sober (14). 3-Methyl-2-benzothiazolone hydrazide hydrochloride was purchased from Aldrich Chemical Company, Inc., Wis. Δ1-Piperideine-2-carboxylic acid was synthesized from o-N-carboxyanilide-1-lysine (15). Δ1-Piperideine-6-carboxylic acid was prepared from L-lysine with the crystalline L-lysine-o-ketoglutarate d-amino transferase (16). o-N-Acetyl-lysine and o-chloro D-alanine were kindly provided by Dr. Morino, Kumamoto University, Kumamoto, Japan. Sodium lauryl sulfate was a specially prepared reagent for protein research (Nakarai Chemicals) and urea was purified by recrystallization from ethanol before use. The other chemicals were analytical grade reagents.

**Enzyme Preparation**—The enzyme was purified from a cell-free extract of *Bacillus sphaericus* IFO 3525 and crystallized as described previously (13), but with higher yield (about 30%). The specific activity of the crystalline preparation was approximately 110.

**Enzyme Assay**—The standard assay mixture consisted of 25 μmol of d-alanine or other d-amino acid, 80 μmol of potassium phosphate buffer (pH 8.0), 1 μmol of pyridoxal 5'-P, 25 μmol of o-keto acid, and 1 μmol of pyridoxal-5'-P, and 25 μmol of a-keto acid, respectively. The Michaelis constants are as follows: D-alanine (1.3 and 4.2 mM with D-alanine, D-a-aminobutyrate and D-glutamate, and a-keto acids, pyruvate, and o-keto acid, respectively). The enzyme activity is significantly affected by both the carbonyl and sulf-hydryl reagents. The Michaelis constants are as follows: d-alanine (1.3 and 4.2 mM with a-keto acid and o-keto acid, respectively), α-keto acid (14 mM with d-alanine), α-keto acid (3.4 mM with d-alanine), pyridoxal 5'-phosphate (2.3 μM) and pyridoxamine 5'-phosphate (25 μM).

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1 The abbreviations used are: pyridoxal-5'-P, pyridoxal 5'-phosphate; pyridoxamine-5'-P, pyridoxamine 5'-phosphate; pyridoxine 5'-P, pyridoxine 5'-phosphate.
enzyme in a final volume of 1.0 ml. Enzyme was replaced by water in a blank. The reaction was initiated by addition of D-amino acid, and incubation was carried out at 37°C for 20 min. The enzyme was assayed by determining pyruvate or amino acid formed with salicylaldehyde as follows, or with ninhydrin, after addition of 0.1 ml of 50% trichloroacetic acid and separation by paper chromatography (17), respectively.

**Determination of Pyruvate**—Pyruvate was determined by a modification of Berntsson's method (18). The reaction was stopped by addition of 1.0 ml of 60% KOH. A 0.5-ml portion of 2% salicylaldehyde in 90% ethanol was added and incubation was performed at 37°C for 30 min to develop an orange color. After addition of 1.5 ml of cold water, the absorbance was measured at 480 nm.

**Protein Determination**—Protein concentration of the enzyme was determined by the method of Lowry et al. (19), as modified by Witkop (20). The absorbance coefficient was 77,000, 16,000, and 7,600, respectively. Fluorescence measurements were performed with a Shimadzu spectrofluorometer type RF 502 with a 1.0-cm light path, and intensities of emission and excitation spectra were given arbitrarily.

**Spectral Measurements**—Absorption and circular dichroism spectra were taken with a Shimadzu MPS-50 L recording spectrophotometer and a Jasco ORD/UV-5 recording spectropolarimeter equipped with a circular dichroism attachment with a 1.0-cm light path, respectively. Fluorescence measurements were performed with a Shimadzu spectrofluorophotometer type RF 502 with a 1.0-cm light path, and intensities of emission and excitation spectra were given arbitrarily.

**RESULTS**

**Molecular Weight**—The molecular weight of D-amino acid aminotransferase was determined to be 58,000 ± 2,000 by sedimentation equilibrium method as reported in a previous paper (13). A molecular weight of about 60,000 was also obtained by the Bio-gel P-150 gel filtration method of Andrews (19), with bovine serum albumin (M, 68,000), ovalbumin (M, 45,000), and α-chymotrypsinogen A (M, 25,000) as standard proteins.

**Structure of Subunits**—D-Amino acid aminotransferase was found to be completely inhibited by 1% sodium lauryl sulfate in 0.02 M sodium phosphate buffer (pH 7.2), or 8 M urea in 0.02 M potassium phosphate buffer (pH 7.4). No activity was recovered after dialysis against two changes of 1000 volumes of the respective buffers at 4°C. These results suggest that the denaturants cause irreversible conformation changes in the enzyme, possibly with dissociation into subunits.

The subunit structure of the enzyme was examined by disc gel electrophoresis. The enzyme was incubated with 1.0% sodium lauryl sulfate in 0.01 M sodium phosphate buffer (pH 7.2) containing 1.0% 2-mercaptoethanol and 25% glycerol, or with 8 M urea in 0.02 M potassium phosphate buffer (pH 7.4) containing 1.0% 2-mercaptoethanol at 37°C for about 12 hours. The treated enzyme preparations were subjected to electrophoresis in the presence of 0.1% sodium lauryl sulfate (20), or of 8 M urea (Fig. 1). There was a single band of stained protein. To determine the molecular weight of the polypeptide in this band, we ran a series of marker proteins treated in the same manner: ovalbumin (M, 45,000), bovine serum albumin (M, 68,000), glutamate dehydrogenase (M, 50,000), cytochrome c (M, 12,000) and chymotrypsinogen A (M, 25,000). The molecular weight was calculated to be approximately 30,000 from a semilogarithmic plot of molecular weight against mobility, suggesting the enzyme consists of two subunits identical in molecular weight.

**Absorption Spectrum of Enzyme and Reduction with Sodium Borohydride**—The holoenzyme exhibits absorption maxima at 280, 330 and 415 nm at pH 7.4 with molecular absorption coefficients of 77,000, 16,000, and 7,600, respectively (Curve A, Fig. 2). No appreciable spectral shifts occurred by varying the pH (5.5 to 10.0). Spectral shifts were observed in the presence of amino donors. The addition of D-alanine or D-glutamate to the enzyme solution at pH 7.4 caused a decrease in absorbance at 415 nm and an increase at about 330 nm. A similar change was observed also after addition of other amino donors. The absorption spectrum was not influenced substantially by addition of α-ketoglutarate or pyruvate at pH 7.4.

**Reduction with Sodium Borohydride**—The holoenzyme in 0.02 M potassium phosphate buffer (pH 7.4) was desalted by column chromatography of Sephadex G-25 with deionized water at 4°C. Resolution of the enzyme did not occur during gel filtration. The enzyme was treated with 50 mM sodium borohydride at 4°C for 10 min by the dialysis method of Matsuo and Greenberg (21), and was then dialyzed against 0.01 M potassium phosphate buffer (pH 7.4).

Spectral measurements showed that the 415 nm peak disap-
peared with enhancement of absorption peak at about 330 nm (Curve B, Fig. 2). This peak did not change after further dialysis against the above-mentioned buffer at 4°C for 18 hours. Reduced enzyme was catalytically inactive, and the addition of pyridoxal-5'-P did not reverse the inactivation. These results suggest that the borohydride reduces the aldimine linkage (λ̇max 415 nm) formed between the 4-aldehyde group of pyridoxal-5'-P and an amino group of the protein to yield the alamine bond (λ̇max 330 nm). An attempt was made to identify the amino residue to which pyridoxal-5'-P binds in the enzyme. The enzyme reduced with sodium borohydride was hydrolyzed in 6 N HCl at 110°C for 24 hours in an evacuated and sealed tube. The hydrolysate was evaporated thoroughly to remove HCl and the residue was dissolved in a small volume of water. An aliquot of the solution was examined by paper electrophoresis in 0.2 M borate buffer 4°C at 1200 volts for 1 hour. A fluorescent spot migrated at the same distance as authentic α-N-pyridoxyllysine, although it was separated from α-N-pyridoxyllysine.

**Resolution and Reconstitution of Enzyme—Pyridoxal-5'-P was not required for maximum activity of the enzyme even after dialysis or Sephadex G-25 gel filtration. The enzyme was only partially resolved when incubated with 0.1 M d-alanine in the presence of 1.0 M phosphate at pH 5.0 (13), although the spectral change was observed as described above. These findings suggest that pyridoxal-5'-P, and also pyridoxamine-5'-P, are tightly bound to the protein moiety of the enzyme. Resolution of the enzyme was performed by treatment with phenylhydrazine as follows. The enzyme was incubated with 5 mM phenylhydrazine (pH 7.4) at 37°C for 1 hour, and then applied to a Sephadex G-25 column (1.5 × 50 cm) equilibrated with 50 mM potassium phosphate buffer (pH 7.4), followed by elution with the same buffer at 4°C. The enzyme thus treated had no activity in the absence of added pyridoxal-5'-P or pyridoxamine-5'-P. Activity is fully restored by addition of either pyridoxal-5'-P or pyridoxamine-5'-P. Pyridoxal-, pyridoxamine-, and pyridoxine-5'-P neither restore activity nor act as inhibitors. The Michaelis constants were estimated as 2.3 μM and 25 μM for pyridoxal-5'-P and pyridoxamine-5'-P, respectively, when the d-alanine and α-ketoglutarate system was employed.

The inactive form of enzyme exhibits no absorption peak at 415 nm, but has a peak at 330 nm (Curve C, Fig. 2). The active enzyme reconstituted with pyridoxal-5'-P shows the spectrum of the native holoenzyme.

**Pyridoxal-5'-P Content and Semiapoenzyme**—The pyridoxal-5'-P content of the holoenzyme was determined by the phenylhydrazine method (22), to be 2 mol per mol of enzyme as reported in a previous paper (13). The enzyme-bound pyridoxal-5'-P was analyzed in duplicate experiments by the phenylhydrazine method (23), after the enzyme solution (1.7 mg in 0.5 ml) was desalted through a Sephadex G-25 column gel filtration with deionized water, and treated with 1.0 ml of 0.1 N HCl at 37°C for 30 min to release the bound pyridoxal-5'-P. An average value of 1 mol of pyridoxal-5'-P per 30,000 g of protein was obtained. This result confirms the cofactor content obtained with phenylhydrazine. The inactive form of enzyme obtained by treatment with phenylhydrazine was examined by both methods in the same way to determine the bound pyridoxal-5'-P absorbing at 330 nm. The results indicate that the inactive form of enzyme still contains 1 mol of pyridoxal-5'-P per mol of enzyme protein. Thus the inactive enzyme is regarded as a "semiapoenzyme" as reported by us, for L-lysine-α-ketoglutarate α-aminotransferase of *Achromobacter liquidum* (24).

The 330 nm peak of semiapoenzyme was never shifted by addition of amino donors or amino acceptors, e.g., d-alanine or α-ketoglutarate, nor by varying the pH of enzyme solution in the range of 5.5 to 10.0, although it disappeared when the enzyme was incubated with 1% sodium lauryl sulfate, 6 M guanidine hydrochloride, 8 M urea, 0.1 N HCl, or 0.1 N NaOH at room temperature for several hours. The holoenzyme and the semiapoenzyme were reduced with sodium borohydride as described above, and treated with 6 M guanidine hydrochloride at 37°C for about 12 hours. After guanidine HCl was removed from the enzyme by gel filtration through a Sephadex G-25 column with 0.05 M potassium phosphate buffer (pH 7.4) at room temperature, the absorption spectra of the treated enzymes were measured. The reduced and denatured form of holoenzyme showed an absorption peak at 330 nm, but the semiapoenzyme treated in the same way exhibited no peak at 330 nm, as shown in Fig. 3. One mole of pyridoxal-5'-P was determined per mol of reduced holoenzyme and semiapoenzyme by the method described above. These results indicate that the bound pyridoxal-5'-P with a 330 nm peak cannot be reduced with sodium borohydride under the conditions used.

**Fluorescence Spectra**—The fluorescence spectra of the enzyme were measured at pH 7.4 (Fig. 4). The holoenzyme
emitted fluorescent light with a maximum at 380 nm and a shoulder at 340 nm upon irradiation at 280 nm. The maximum was shifted to 465 nm on excitation at 415 nm. When the enzyme was analyzed at 380 nm, excitation spectra exhibited maxima at 270, 290, and 330 nm (Fig. 5). The same excitation spectrum was obtained with the semiaepoenzyme. The emission spectrum of the semiaepoenzyme was the same as that of the holoenzyme when excited at 330 nm, although the semiaepoenzyme did not emit fluorescence upon excitation at 415 nm.

**Circular Dichroism Spectra**—Circular dichroic spectra of the holoenzyme, and the holoenzyme reduced with NaBH₄ and denatured with 6 M guanidine hydrochloride, were measured at pH 7.4 (Fig. 6). The native holoenzyme spectrum in the visible region is optically active. A positive circular dichroic extremum at 280 nm and negative ones at 330 and 415 nm correspond to the positions of absorption maxima. The reduced and denatured enzyme showed no circular dichroism band at these wavelengths, suggesting that the bound phosphopyridoxyl moiety in subunits is no longer optically active.

**Substrate Specificity**—The ability of the enzyme to catalyze transamination between various amino acids and α-ketoglutarate, pyruvate, or α-keto-βutyrate was re-examined and is presented in Table I. D-Alanine, D-α-amino-βutyrate, D-ethionine, D-norvaline and D-glutamate are good amino donors for these keto acids. D-Lysine and D-ornithine, diamino monobasic acids, also serve as amino donors to a lesser extent. The transamination product from D-lysine was investigated in a pyruvate system. The product reacted with α-aminobenzaldehyde and ninhydrin to develop an orange and a yellow color, respectively, in the same manner as reported previously (16), suggesting that a heterocyclic form of the keto analogue of lysine, i.e. Δ¹-piperideine-2-carboxylate or Δ¹-piperideine-6-carboxylate, is produced. The reaction mixture containing D-lysine, pyruvate, and 10 μg of enzyme was incubated at 30°C for 60 min. After deproteinization by addition of 0.2 ml of 10% trichloroacetic acid, followed by centrifugation, the supernatant was adjusted to about pH 2.0, and analyzed by a cation exchange chromatography (Fig. 7). The product from D-lysine was identified with authentic Δ¹-piperideine-2-carboxylate prepared from α-N-carboxy-L-lysine. This result indicates the occurrence of α-transamination between D-lysine and pyruvate to produce Δ¹-piperideine-2-carboxylate.

Amino acceptor specificity also was investigated in the reaction system containing D-alanine as an amino donor (Table I). α-Ketobutyrate, α-ketoglutarate, and α-ketovalerate are the preferred amino acceptors. All L-amino acids tested, and glycine, are inert.

**Kinetics**—The Michaelis constants for D-alanine were determined to be 1.3 and 4.2 mM in transamination with α-keto-βutyrate and α-ketoglutarate, respectively, according to the method of Velick and Vavra (25). The constants for α-keto-βutyrate and α-ketoglutarate were calculated to be 14 and 3.4 mM, respectively, when D-alanine was used as an amino donor.

**Inhibitors**—The various compounds were investigated for their inhibitory effects on enzyme activity (Table III). Phenylhydrazine, hydroxylamine, aminoxyacetate, 3-methyl-2-benzoxazolyl, etc., were effective inhibitors.

![Fig. 5. Excitation spectrum of L-amino acid aminotransferase. Excitation spectrum of holoenzyme analyzed at 380 nm was measured under the same conditions as described in Fig. 4. Intensity is given as uncorrected values.](http://www.jbc.org/)

![Fig. 6. Circular dichroic spectra of L-amino acid aminotransferase. Spectra of the native (I) holoenzyme and the reduced-denatured (II) holoenzyme (4.8 mg/ml) were measured in 0.02 M potassium phosphate, pH 7.4, in a cuvette at 25°C.](http://www.jbc.org/)

<table>
<thead>
<tr>
<th>Amino acids</th>
<th>α-Ketoglutarate</th>
<th>Pyruvate</th>
<th>α-Keto-βutyrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>D-Alanine</td>
<td>100</td>
<td>100</td>
<td>62</td>
</tr>
<tr>
<td>D-Glutamate</td>
<td>52</td>
<td>45</td>
<td>30</td>
</tr>
<tr>
<td>D-α-Amino-βutyrate</td>
<td>97</td>
<td>118</td>
<td></td>
</tr>
<tr>
<td>D-Glutamine</td>
<td>31</td>
<td>28</td>
<td>16</td>
</tr>
<tr>
<td>D-Asparagine</td>
<td>40</td>
<td>25</td>
<td>20</td>
</tr>
<tr>
<td>D-Threonine</td>
<td>30</td>
<td>24</td>
<td>21</td>
</tr>
<tr>
<td>D-β-Arbutate</td>
<td>61</td>
<td>62</td>
<td>50</td>
</tr>
<tr>
<td>D-Methionine</td>
<td>76</td>
<td>67</td>
<td>46</td>
</tr>
<tr>
<td>D-ε-Thionine</td>
<td>83</td>
<td>61</td>
<td>46</td>
</tr>
<tr>
<td>D-Norvaline</td>
<td>46</td>
<td>33</td>
<td>22</td>
</tr>
<tr>
<td>D-Leucine</td>
<td>8</td>
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<td>8</td>
</tr>
<tr>
<td>D-Tryptophan</td>
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<td>9</td>
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<tr>
<td>D-Phenylalanine</td>
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<td>18</td>
<td>10</td>
</tr>
<tr>
<td>D-Arginine</td>
<td>3</td>
<td>8</td>
<td>7</td>
</tr>
<tr>
<td>D-Lysine</td>
<td>16</td>
<td>7</td>
<td>5</td>
</tr>
<tr>
<td>D-Ornithine</td>
<td>6</td>
<td>2</td>
<td>2</td>
</tr>
</tbody>
</table>


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Table I

**Amino donor specificity**

The enzyme activity was assayed by determining alanine, glutamate, or α-aminobutyrate formed with an automatic amino acid analyzer (Yanagimoto LC-5S). The relative activity less than 0.5 is expressed as zero.
Fig. 7. Ion exchange chromatography of the reaction product from D-lysine. The reaction mixture, containing 50 μmol of D-lysine, 50 μmol of pyruvate, 1 μmol of pyridoxal 5'-P, 80 μmol of potassium phosphate (pH 8.0), and 10 μg of enzyme in a final volume of 2.0 ml, was incubated at 37°C for 60 min. After deproteinization by addition of 0.2 ml of 50% trichloroacetic acid, followed by centrifugation, the supernatant was adjusted to about pH 2.0 and applied to an Aminex A-4 column (0.8 x 55 cm) equilibrated with 0.2 M citrate buffer. The column was eluted with the same buffer at the flow rate of 1.7 ml/min at 55°C. The eluate was collected in 2.5-ml portions and mixed with 0.5 ml of 0.04 M o-aminobenzaldehyde solution. Absorbance was measured at 465 nm after incubation at 37°C for 12 hours. A, authentic Δ1-piperideine-2-carboxylate (P-2-C) and Δ1-piperideine-6-carboxylate (P-6-C); B, reaction product.

TABLE II

Amino acceptor specificity

The enzyme activity was determined in the reaction system containing D-alanine as an amino donor by a salicylaldehyde method.

<table>
<thead>
<tr>
<th>Keto acids</th>
<th>Relative activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-Ketoglutarate</td>
<td>100</td>
</tr>
<tr>
<td>α-Ketobutyrate</td>
<td>100</td>
</tr>
<tr>
<td>α-Ketovalerate</td>
<td>88</td>
</tr>
<tr>
<td>α-Ketocaproate</td>
<td>61</td>
</tr>
<tr>
<td>Oxalacetate</td>
<td>57</td>
</tr>
<tr>
<td>Glyoxylate</td>
<td>34</td>
</tr>
<tr>
<td>Phenylpyruvate</td>
<td>12</td>
</tr>
<tr>
<td>α-Ketoisocaproate</td>
<td>11</td>
</tr>
<tr>
<td>α-Ketoisovalerate</td>
<td>3</td>
</tr>
</tbody>
</table>

Zothiazolone hydrazone hydrochloride, D-cycloserine, and D- and L-penicillamine, which are typical inhibitors for pyridoxal 5’-P enzymes, strongly inhibit the enzyme. D-Cycloserine, and D- and L-penicillamine function as a competitive inhibitor against D-alanine, and the following inhibition constants were determined in transamination between D-alanine and α-ketoglutarate: D-cycloserine (11 μM), D-penicillamine (57 μM), and L-penicillamine (0.8 μM). β-Chloro-D-alanine also competes against D-alanine to decrease activity (Kₐ = 2.25 μM).

Thiol reagents, e.g. HgCl₂ and N-ethylmaleimide, significantly inhibited the enzyme. The enzyme was protected about 68% from the inhibition with HgCl₂ by D-alanine or α-ketoglutarate.

DISCUSSION

Molecular asymmetry plays an important role in enzymatic specificity and dictates the spatial architecture of various biological polymers. The concepts of protein and amino acid biochemistry have grown mainly out of observations and knowledge of L-amino acids. Recently a variety of D-amino acids, however, have been demonstrated in both the free state and peptide linkages in microorganisms, higher plants, and animals as reviewed by Meister (26) and Corrigan (27). There has been increasing interest in the properties and physiological functions of D-amino acid aminotransferase, a key enzyme of D-amino acid metabolism. D-Amino acid aminotransferase has been purified to homogeneity from the extract of Bacillus sphaericus to permit us to study accurately the enzymological and physicochemical properties and the reaction mechanism (13).

The enzyme is of very high optical specificity for the D-enantiomer of amino acids. Although L-amino acid aminotransferases have generally high structural substrate specificity (28–31), D-amino acid aminotransferase is characterized by its low substrate specificity. Most of the D-amino acids tested are or are less transaminated by the enzyme with α-ketoglutarate, pyruvate, and α-ketobutyrate with some exceptions. In general, the good amino donors have an ω-carboxyl group, such as, or a hydrophobic unbranched side chain, e.g. D-glutamate, D-theanine (γ-D-glutamylethylamide), and D-α-amino butyrate. The presence of a hydroxyl group prevents probably the D-amino acids from binding with the enzyme, since D-serine, D-threonine, and D-tyrosine cannot be the substrates. Glycine is inert as either substrate or inhibitor, while α-ketoglutarate is transaminated with some D-amino acids. There is some difference between substrate specificity of D-alanine-D-glutamate aminotransferase of B. subtilis (10) and that of B. sphaericus. The B. subtilis enzyme catalyzes transamination between D-serine and α-ketoglutarate, and D-lysine and D-phenylalanine are inert as amino donor, whereas the B. sphaericus enzyme does not transaminate D-serine, but does D-lysine and D-phenylalanine with α-ketoglutarate, pyruvate, or α-ketobutyrate.

TABLE III

Effect of inhibitors on transamination between D-alanine and α-ketoglutarate

After the enzyme was previously incubated with the compounds at 37°C for 15 min, the activity was determined by a salicylaldehyde method. All the compounds listed did not interfere with the assay.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Concentration</th>
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<tr>
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<tr>
<td>D-Cysteine</td>
<td>1</td>
<td>95</td>
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<tr>
<td>L-Cysteine</td>
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<td>93</td>
</tr>
<tr>
<td>D-Cycloserine</td>
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<td>Aminoxacetic acid</td>
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</tr>
<tr>
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<td>MBTH¹</td>
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</tr>
<tr>
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</tr>
<tr>
<td>β-Cl-D-Alanine</td>
<td>0.025</td>
<td>44</td>
</tr>
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</table>

¹MBTH, 3-methyl 2-benzothiazolone hydrazone hydrochloride; PCMB, p-chloromercuribenzoate.
An \( \alpha \)-amino group of \( \beta \)-lysine is transferred in the enzyme system. The bacterial \( \beta \)-lysine aminotransferases so far studied catalyze \( \epsilon \) transamination of lysine to produce \( \Delta^1 \)-piperideine-6-carboxylate (16, 32), whereas \( \beta \)-lysine aminotransferase of pea seedlings transaminates \( \beta \)-lysine at the \( \alpha \) position (32). The result obtained here provides the first example for the \( \alpha \) transamination of lysine by a bacterial enzyme. It is suggested that the enzyme transaminates \( \beta \)-ornithine also at \( \alpha \) position by analogy with the lysine transamination. We reported previously that arginine racemase of \textit{Pseudomonas graveolens} catalyzes racemization of various amino acids and \( \alpha \) transamination between \( \beta \)- and \( \alpha \)-ornithine, and pyruvate as well to regulate the enzyme activity (33).

The enzyme is competitively inhibited by either \( \beta \)- or \( \epsilon \)-penicillamine, but \( \beta \)-penicillamine has a higher affinity for the enzyme than the \( \epsilon \)-enantiomer. It is likely that the \( \beta \)-enantiomer is less hindered sterically as well as the substrate \( \beta \)-amino acids to bind the active site of the enzyme. This \( \beta \)-specific configuration of the active site may be reflected on the negative circular dichroism band about at 415 nm, which is derived from the bound pyridoxal-5'-P. All of the circular dichroic spectra of \( \beta \)-amino acid aminotransferases thus far studied in this region are positive (34, 35). It was reported that \( \beta \)-alanine-\( \beta \)-glutamate aminotransferase of \textit{B. subtilis} is not sensitive to sulfhydryl reagents, whereas the enzyme of \textit{B. sphaericus} is inhibited by the reagents, and the inhibition is diminished by preincubation with either amino donor or acceptor, suggesting that a sulfhydryl group of the enzyme plays a significant role in the catalytic action.

The holoenzyme (\( \lambda_{\text{max}} \) 330 and 415 nm) and the semiaopenzyme (\( \lambda_{\text{max}} \) 330 nm) contain 2 and 1 mol of pyridoxal-5'-P, respectively. It is suggested that the catalytic activity of the enzyme is concerned with only 1 mol of the bound pyridoxal 5'-P with an absorption maximum at 415 nm, which is converted into the pyridoxamine-5'-P by incubation with the amino donor. Martinez-Carrion and Jenkins (10) reported that \( \beta \)-alanine-\( \beta \)-glutamate aminotransferase of \textit{B. subtilis} which also shows absorption maxima at 350 and 415 nm contains 1 mol of pyridoxal-5'-P per mol of enzyme, and that it is resolved to apoenzyme by incubation with an amino donor in the presence of high concentration of phosphate. The enzyme of \textit{B. sphaericus} is only partially resolved by the same treatment (13), suggesting that the \textit{B. sphaericus} enzyme is bound more tightly with pyridoxamine-5'-P than the \textit{B. subtilis} enzyme.

After denaturation with guanidine HCl, the semiaopenzyme reduced with sodium borohydride does not have an absorption peak at 330 nm, but the reduced holoenzyme does. This indicates that the aldamine linkage (\( \lambda_{\text{max}} \) 330 nm) formed between pyridoxal-5'-P and an \( \epsilon \)-amino group of lysine residue of protein is not influenced by treatment with the denaturant, and also that the 330 nm bound pyridoxal-5'-P is not released with sodium borohydride under the conditions employed and is released from the protein by the denaturation. This bound pyridoxal-5'-P seems to be similar to that found in the semiaiform of \( \beta \)-lysine-\( \epsilon \)-ketoglutarate \( \epsilon \)-aminotransferase (24). The occurrence of the pyridoxal-5'-P with absorption maximum about at 330 nm has been reported also for some other pyridoxal-5'-P enzymes, e.g. phosphorylase b (36) and glutamate decarboxylase of \textit{E. coli} (37). Three possible structures have been proposed for the 330 nm bound pyridoxal-5'-P: an enol imine form (38, 39) and a carbinal amine form (40) in a hydrophobic pocket, and a substituted aldamine form (41), although unambiguous evidence for the structures has not been obtained. The bound pyridoxal-5'-P of glutamate decarboxylase absorbing at 340 nm in the neutral pH range is not susceptible to sodium borohydride reduction, and exhibits fluorescence maximum at 380 nm on irradiation at 340 nm (37). \( \beta \)-Amino acid aminotransferase of \textit{B. sphaericus} and glutamate decarboxylase are resemble each other in these respects. But, the 540 nm bound pyridoxal-5'-P of glutamate decarboxylase is not optically active, whereas that of \( \beta \)-amino acid aminotransferase exhibit a negative CD band in the 300-nm region. The optical inactivity of the glutamate decarboxylase bound pyridoxal-5'-P was ascribed to formation of an achiral aldamine between the Schiff base and an \( \epsilon \)-amino group of the second lysine residue of protein (41). If this is the case also for the aminotransferase, the optical active 330 nm pyridoxal-5'-P may be attributable to a chiral aldamine formed between the Schiff base and a hydroxyl, sulfhydryl, imidazole, or indole group of protein (42, 43). Recently O’Leary and Brummond observed the slightly optical activity of the 340 nm pyridoxal-5'-P of glutamate decarboxylase and suggested that at high pH a sulfhydryl group of the enzyme may add to the Schiff base forming an aldamine (44).

The enzyme consists of two subunits identical in molecular weight. It is likely that one of them binds to 1 mol of pyridoxal-5'-P to form an internal Schiff base and to participate directly in catalytic action. The other subunit also binds to one mole of pyridoxal-5'-P whose role is unknown in a different way. The structure of the subunits is now under investigation in detail from the standpoint of protein chemistry.

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