Guanidinobutyrase for L-Arginine Degradation in Brevibacterium helvolum

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Guanidinobutyrase (guanidinobutyrate amidinohydrolase, EC. 3.5.3.7) catalyzing the third step of the arginine oxygenase pathway in Brevibacterium helvolum IFO 12073 (ATCC 11822) was purified to homogeneity and characterized. The enzyme had a molecular weight of 190,000 and was composed of four apparently identical subunits with a molecular weight of 45,000. The E(1%)/lcm value at 280 nm of the enzyme protein was 2.4. The enzyme contained 0.5 mol of firmly bound Zn²⁺ per mol of subunit. The enzyme was highly specific for 4-guanidinobutyrate, but had a weak activity toward L- and D-arginine. The Michaelis constant (Kₘ) for 4-guanidinobutyrase was 2.9 mM. The optimum pH was 9.0. Strong mixed type inhibition was observed with thiglycolate and several other thiol compounds. These properties were compared with those of the enzyme of fluorescent Pseudomonas and discussed.

Bacterial guanidinobutyrase (EC 3.5.3.7) has been found in fluorescent Pseudomonas strains.⁴⁻⁶ Bacterial strains and culture methods. Brevibacterium helvolum IFO 12073 (ATCC 11822), B. ammonigenes IFO 12072 (ATCC 6872), B. stationis IFO 12144, and B. linens IFO 12141 were obtained from the Institute for Fermentation (Osaka, Japan). For the study of the distribution of guanidinobutyrase, cells were grown in the basal medium⁵ containing monosodium succinate, disodium succinate, and 4-guanidinobutyrate at concentrations of 0.01, 0.09, and 0.1%, respectively. Cells of B. helvolum used for enzyme preparation were grown and exposed to L-arginine in 15-liters batches as described in our previous paper except that the peptone-glycerol medium had 50 µM of ZnSO₄ added. Other culture conditions are given in our previous paper. About 80 g of cell paste was obtained from a 15-liters batch culture.

Assay of guanidinobutyrase. The standard assay conditions for guanidinobutyrase and the definition of the enzyme unit are given in our previous paper. The enzyme reaction was allowed to proceed at 30°C for 30 min in 100 mM Tris-hydrochloride buffer, pH 9.0, in the presence of 4-guanidinobutyrate at a concentration of 25 mM, and then the urea formed was measured colorimetrically. The activity of the enzyme toward L-arginine was measured under the same conditions except that L-arginine was added at a concentration of 50 mM.

Analytical methods. L-Arginine, 4-guanidinobutyrate, urea, and protein in enzyme preparations were measured by the methods described in our previous paper. Protein in homogeneous preparations of guanidinobutyrase was measured from the absorbance at 280 nm using the E(1%)~/cm value of 2.4, which was obtained from the absorption at 280 nm and the approximate weight-based protein concentration of a purified enzyme solution. The protein concentration was measured refractometrically using an HPLC system as described in our previous paper; the system was calibrated with bovine serum albumin.

Measurement of molecular weights. The molecular weight of undenatured guanidinobutyrase was measured by an HPLC procedure with a TSK-GEL G3000SW column (0.75 x 30 cm). The molecular weight standards were ferritin (45), glutamate dehydrogenase (28), aldolase (15.8), bovine serum albumin (6.8), egg albumin (4.5), and cytochrome c (1.25); the value in the parenthesis indicates 1/10³ of the molecular weight of each protein.

The molecular weight of the subunit of the enzyme and its oligomers were measured by SDS-polyacrylamide gel electrophoresis with both.
continuous and discontinuous buffer systems. The enzyme protein, 0.11 mg, was incubated in 100 μl of 10 mM potassium phosphate buffer, pH 8.0, containing 15% acetone and 2.5 mM 3,3'-dithiobis (propionic acid N-hydroxysuccinimide ester) at 30°C for 24 hr. The cross-linking reaction was stopped by adding 20 μl of 20 mM potassium phosphate buffer, pH 8.0, containing 50 mM ethanolamine and 20 mM N-ethylmaleimide. The mixture was mixed with 60 μl of 125 mM Tris–hydrochloride buffer, pH 8.0, containing 3% SDS, and the solution was heated at 100°C for 5 min. The sample for electrophoresis was prepared by adding to the mixture the same volume (180 μl) of 50% glycerol containing 0.01% bromphenol blue.

### Polycrylamide gel electrophoresis (PAGE). Undenatured enzyme was electrophoresed in a slab gel containing 7.5% polyacrylamide according to the method of Davis.11 SDS PAGE with a discontinuous buffer system was done by the method of Laemmli,12 and that with a continuous buffer system was done in a slab gel containing 5% polyacrylamide, 0.2% SDS, 50 mM Tris, and 380 mM glycine. The protein bands in the gel were stained in 50% methanol containing 10% acetic acid and 0.1% Coomassie Brilliant Blue G250. The monomer and its oligomers of cytochrome c were used as the molecular weight standards.

### Measurement of zinc and cobalt ions in enzyme protein. A solution, 1.34 mg/ml (6.96 nmol/ml), of purified guanidinobutyrate dialyzed against 5.0 mM Tris–hydrochloride buffer, pH 9.0, was used as the sample. An atomic absorption spectrophotometer, Shimazu AA-640-01, was calibrated with the same buffer containing 1.0 mg/ml of egg albumin and below 1.0 ppm of Zn²⁺ or Co²⁺. Wavelengths of 215.9 and 240.7 nm were used for Zn²⁺ and Co²⁺, respectively.

### Purification of guanidinobutyrase. The following operation were done below 5°C unless stated otherwise. Cells of B. helvolum, 270 g (wet weight), were suspended in 540 ml of 20 mM potassium phosphate buffer, pH 8.0, and disrupted with a sonic disintegrator at 5–10°C. The supernatant solution (420 ml) obtained by centrifugation at 28,000 × g for 20 min was diluted with 420 ml of the buffer. The crude extract was brought to 20% saturation with ammonium sulfate and the precipitate formed was removed by centrifugation. The supernatant was brought to 50% saturation with ammonium sulfate and stirred gently for 90 min. The precipitated protein was dissolved in the buffer and dialyzed thoroughly against the buffer containing 0.15 M KCl.

The ammonium sulfate fraction (362 ml) was separated into two portions and each portion was put on a DEAE-Toyopearl 650 M column (4.5 × 25 cm) equilibrated with the buffer containing 0.15 M KCl. The column was washed with 2,000 ml of the equilibration buffer, and then the enzyme was eluted with the buffer containing 0.18 M KCl. The active fractions were pooled (2510 ml).

Ammonium sulfate was added to the enzyme to a concentration of 1.0 M, and the enzyme was put on a Butyl Toyopearl 650 M column (3 × 17 cm) equilibrated with 50 mM potassium phosphate buffer, pH 8.0, containing 1.0 M ammonium sulfate. The enzyme was eluted by a linear gradient of ammonium sulfate made by 700 ml of 50 mM potassium phosphate buffer, pH 8.0, containing 1.0 M KCl placed in a reservoir and the same volume of the buffer without KCl placed in a mixing bottle; 10-ml fractions were collected. The protein elution profile showed three peaks and the second peak showed the activity. The active fractions were pooled (150 ml) and concentrated by ultrafiltration to about 2 ml.

The enzyme was put on a Toyopearl HW-55F column (1.0 × 123 cm) equilibrated with 20 mM potassium phosphate buffer, pH 8.0, containing 0.2 M KCl, and then eluted with the equilibration buffer; 1-ml fractions were collected. The protein elution showed a single peak. Fractions from 49 to 52, which showed almost equal specific activities, were pooled. The enzyme (4 ml) was brought to 25% (w/v) with glycerol and stored in a freezer at -20°C.

### Results

#### Purification of guanidinobutyrase and molecular properties of enzyme

A typical purification profile of guanidinobutyrase of B. helvolum is shown in Table I. The enzyme was purified with an yield of 14%. The E(1%) value at 280 nm of the enzyme protein in the final preparation was measured to be 2.4 by a refractometric method.19 On the basis of this value, the amount of protein and the specific activity of the final preparation were calculated to be 12.4 mg and 83.8 units/mg, respectively, indicating an 195-fold purification.

The purified enzyme showed a single protein band on polyacrylamide gel electrophoresis in the presence or absence of SDS. Molecular sieve chromatography of the enzyme with an HPLC column gave the molecular weight of the enzyme as 194,000 (Fig. 1a). The subunit molecular weight was 45,000 on SDS–PAGE of the denatured enzyme (Fig. 1b). The oligomers of the subunits prepared by cross-linking with 3,3'-dithiobis(propionic acid N-hydroxysuccinimide ester) gave four major bands, but their molecular weights could not be measured because of their low mobilities (Fig. 2a). Their molecular weights were estimated by SDS–PAGE with a continuous buffer system. As shown in the lane D in Fig. 2b, four bands of the monomer and the cross-linked enzyme protein were found. Bands 3 and 4 were broadened during electrophoresis; this may be caused by the sample containing various cross-linked forms including straight chains, triangles, and so on. The bands with the largest and smallest mobilities had molecular weights of 44,000 and 210,000 respectively; the latter value was close to the

#### Table 1. Purification of Guanidinobutyrase

<table>
<thead>
<tr>
<th>Step</th>
<th>Protein (mg)</th>
<th>Activity (units)</th>
<th>Sp. activity (units/mg)</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell extract</td>
<td>16,800⁰</td>
<td>7,200</td>
<td>0.43</td>
<td>100</td>
</tr>
<tr>
<td>Ammonium sulfate</td>
<td>7,830²</td>
<td>5,800</td>
<td>0.74</td>
<td>81</td>
</tr>
<tr>
<td>DEAE-Toyopearl</td>
<td>200³</td>
<td>3,460</td>
<td>17.3</td>
<td>48</td>
</tr>
<tr>
<td>Butyl-Toyopearl</td>
<td>7.3⁴</td>
<td>1,980</td>
<td>271</td>
<td>28</td>
</tr>
<tr>
<td>Toyopearl HW-55F</td>
<td>12.4⁵</td>
<td>1,044</td>
<td>83.8</td>
<td>14</td>
</tr>
</tbody>
</table>

¹ Measured by the method of Lowry et al. ¹⁰
² Measured from A₅₆₀ using an E(1%) value of 10.0.
³ Measured from A₅₆₀ and the E (1%) of 2.4. The E(1%) value was obtained by the refractometric measurement of the enzyme protein.

![Fig. 1. Measurements of Molecular Weight and Subunit Size of Enzyme](image-url)

- a: The sample, 25 μl was transferred to a TSK-GEA G3000SW column (0.75 × 30 cm) with 0.1 M potassium phosphate buffer, pH 7.0, containing 0.2 M KCl and 0.05% sodium azide at a flow rate of 1.0 ml/min and the absorbance of the eluate was measured at 280 nm. The standard proteins were: 1. ferritin; 2. glutamate dehydrogenase; 3. aldolase; 4. bovine serum albumin; 5. egg albumin, 6. chymotrypsinogen A; 7. cytochrome c. The arrow indicates the elution volume of purified guanidinobutyrase.
- b: The sample, 10 μl was put on a slab gel containing 3% polyacrylamide (stacking gel) and electrophoresed into a gel containing 10% polyacrylamide. Other conditions were the same as those described by Laemmli. The relative mobility was calculated from the mobility of bromphenol blue. The standard proteins were the oligomers of cytochrome c: 1. hexamer; 2. tetramer; 3. trimer; 4. dimer; 5. monomer. The arrow indicates the relative mobility of purified guanidinobutyrase subunit.
molecular weight of the undenatured enzyme. These results indicate that the enzyme has a molecular weight of about 190,000 and is composed of four apparently identical subunits with a molecular weight of about 45,000.

When the enzyme was electrophoresed just after mixing with SDS at a room temperature, a protein band with a relative mobility corresponded to a molecular weight of about 65,000 was found (lane B in Fig. 2b). This value was considerably larger than that of the monomer and smaller than that of the dimer. This form of the enzyme protein dissociated to the monomer after heating in boiling water for 2 min (lane C). It seems most likely that this form of the enzyme was a dimer of compactly associated subunits. The faint band in lane C in Fig. 2a may be that of the same form of the enzyme.

Metal content and activation

The enzyme was purified from cells grown in a medium containing Zn\(^{2+}\) at a concentration of 50 \(\mu\)M to avoid the binding of other metal ions. The enzyme of any purification steps gave full activities without added Zn\(^{2+}\). The enzyme activity was lost by 80% on incubation at pH 11.0 at 30°C for 60 min in the presence of 20 mM \(\beta\)-phenanthroline, and the activity was restored to about 50% of the original activity by incubation with Zn\(^{2+}\) or Co\(^{2+}\) at a concentration of 0.1 mM. Mn\(^{2+}\) and Ni\(^{2+}\) had essentially no effect. The enzyme from cells grown in a medium without added Zn\(^{2+}\) gave similar results (data not shown).

Atomic absorption analyses of an enzyme solution containing 7.0 nmol of the enzyme per ml (1.34 mg/ml), which was calculated from the molecular weight of 190,000, gave a result that the enzyme contained 15.4 nmol/ml of Zn\(^{2+}\). No Co\(^{2+}\) was detected in this experiment. This indicates that one mol of the tetrameric enzyme had 2 mol of Zn\(^{2+}\). The enzyme showed 92% of the original activity after incubation with 0.1 mM Zn\(^{2+}\) at 30°C for 10 min. This suggested that the enzyme was saturated with about 0.5 mol of Zn\(^{2+}\) per mol of the subunit. An about 40% inhibition was observed in the presence of 1.0 mM Zn\(^{2+}\).

Substrate specificity

Various guanidino compounds were tested as the substrate of guanidinobutyrase under the standard assay conditions (Table II). The enzyme had a rather high substrate specificity; the next lower and higher homologues of 4-guanidinobutyrate showed very weak activities and guanidinocacetate was inert. However, L-arginine, D-arginine, and L-canavanine was hydrolyzed, although the rates were very low. The activity toward L-arginine (arginase activity) of the crude extract was about the same as that of the purified enzyme.

Stability on heating

The enzyme was heated at 50°C in 20 mM potassium phosphate buffer, pH 8.0, for various times and then the activity of each sample was measured with both 4-guanidinobutyrate and L-arginine as the substrates (Fig. 3). The residual activity after heating for 60 min toward 4-guanidinobutyrate was about 50% of the original en-

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**Table II. Substrate Specificity of Guanidinobutyrase**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Relative activity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3-Guanidinopropionate</td>
<td>0.94</td>
</tr>
<tr>
<td>4-Guanidinobutyrate</td>
<td>100</td>
</tr>
<tr>
<td>5-Guanidinovalerate</td>
<td>1.5</td>
</tr>
<tr>
<td>L-Arginine</td>
<td>0.62</td>
</tr>
<tr>
<td>D-Arginine</td>
<td>2.9</td>
</tr>
<tr>
<td>L-Canavanine</td>
<td>1.0</td>
</tr>
</tbody>
</table>

The following compounds were inert as substrates: guanidinocacetate, L-guanidinosuccinate, 6-guanidinopropionate, 2-guanidinocytochrome c, 5-guanidinobutyramide, 5-guanidinobutyramide, taurine, creatine, L-amino-L-alanine, and agmatine.

* The reaction mixture for 4-guanidinobutyrate contained 0.018 unit of purified guanidinobutyrase and those for other compounds contained 0.93 unit of the enzyme.

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Fig. 2. SDS-PAGE of Guanidinobutyrase with Discontinuous (a) and Continuous (b) Buffer Systems.

- Lane A, SDS-denatured guanidinobutyrase cross-linked with 3,3'-dithiobis(propionamide). The numbers show the positions of the band of the cross-linked enzyme: 1, the monomer; 2, the dimer; 3, the trimer; 4, the tetramer. Lane B, the monomer of horse cytochrome c migrated to the same position as bromphenol blue and its oligomers. Lane C, SDS-denatured guanidinobutyrase. Lane D, cross-linked guanidinobutyrase, the same sample that used for lane A of Fig. 2a.

Fig. 3. Effects of Heat on Activity of Guanidinobutyrase.

Purified guanidinobutyrase, 0.93 unit, was heated in 20 mM potassium phosphate buffer, pH 8.0, at 50°C for the time indicated, and then the activity was measured in the standard reaction mixture containing 25 mM guanidinobutyrate (O) or 0.5 mM L-arginine (●).
zyme activity. The activities of the samples toward L-arginine were almost the same as those toward 4-guanidinobutyrate.

**Effects of pH on activity**

The activity of the enzyme was measured in various buffers at various pHs with 4-guanidinobutyrate and L-arginine as the substrates (Fig. 4). The same optimum pH of 9.0 was obtained with both substrates. The lower activity toward L-arginine at pH below 8.0 may be due to the positive charge of the amino group of the compound.

**Kinetic constants**

The reaction rates at various concentrations of 4-guanidinobutyrate and L-arginine were measured under the standard assay conditions. Lineweaver-Burk plots of the data gave the apparent \( K_m \)s of 2.9 and 71 mM for 4-guanidinobutyrate and L-arginine, respectively. The \( V_{\text{max}} \) for 4-guanidinobutyrate and L-arginine were 98 and 1.1 \( \mu \text{mol/min/mg protein} \), respectively.

**Inhibitors**

The effects of various compounds on the enzyme activity was examined under the standard assay conditions (Table III). \( \omega \)-Amino acids, lower fatty acids, and thiol compounds inhibited the enzyme. The inhibition by 5-aminovalerate and \( n \)-valerate was competitive; the apparent \( K_i \) values were 0.55 and 1.5 mM, respectively (Fig. 5). Many acidic, neutral, and basic SH compounds were strong inhibitors of the enzyme. About 80% inhibition was observed with 0.1 mM thioglycolate. The inhibition with thioglycolate and mercaptoethanolamine was of the mixed type (Fig. 5).

**Guanidinobutyrase in Brevibacterium strains**

In our previous paper we have reported that *Arthrobacter globiformis* IFO 12137 (ATCC 8010), *A. simplex* IFO 12069 (ATCC 6946), *Brevibacterium helvolum* IFO 12073 (ATCC 11822), and *B. lipolyticum* IFO 12678 produce guanidinobutyrase when grown on L-arginine, and that *B. ammoniagenes* IFO 12072 (ATCC 6872), *B. stationis* IFO 12144, and *B. linens* IFO 12141 do not grow on L-arginine but can grow on 4-guanidinobutyrate.

We examined the latter three strains for the ability to produce guanidinobutyrase by measuring the activity of extracts of cells grown on 4-guanidinobutyrate. The extracts of the three strains contained guanidinobutyrase; the specific activities were about 0.1 unit per mg protein.

**Discussion**

Guanidinobutyrase, which catalyzes the third step of the arginine oxygenase pathway, was purified from *B. helvolum* IFO 12073 to homogeneity and characterized. The strain is a member of the group of coryneform bacteria, which is phylogenetically close to the actinomycete group and rather remote to the group of fluorescent *Pseudomonas*. Guanidinobutyrase of *P. putida*, which participates in the arginine oxidase pathway, has been purified and characterized.\(^2\) The *Brevibacterium* enzyme has a subunit molecular weight of 45,000, which is larger than that (about 35,000) of *Pseudomonas* enzyme. The *Pseudomonas* enzyme requires Mn\(^{2+}\), which can be easily dissociated from the enzyme with EDTA. In contrast, the *Brevibacterium* enzyme has firmly bound Zn\(^{2+}\); attempts at

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**Table III. Effects of Various Compounds on Guanidinobutyrase**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Conc. (mM)</th>
<th>Relative activity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>—</td>
<td>100</td>
</tr>
<tr>
<td>Agmatine</td>
<td>5.0</td>
<td>72</td>
</tr>
<tr>
<td>5-Aminovalerate</td>
<td>5.0</td>
<td>67</td>
</tr>
<tr>
<td>6-Aminopropionate</td>
<td>5.0</td>
<td>79</td>
</tr>
<tr>
<td>( n )-Butyrate</td>
<td>5.0</td>
<td>57</td>
</tr>
<tr>
<td>( n )-Valerate</td>
<td>5.0</td>
<td>66</td>
</tr>
<tr>
<td>Crotonate</td>
<td>5.0</td>
<td>60</td>
</tr>
<tr>
<td>Thioglycolate</td>
<td>0.1</td>
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</tr>
<tr>
<td>Thioglycolate</td>
<td>1.0</td>
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</tr>
<tr>
<td>2-Mercaptoethanol</td>
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</tr>
<tr>
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<td>89</td>
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<tr>
<td>Dithioerythritol</td>
<td>5.0</td>
<td>51</td>
</tr>
</tbody>
</table>

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**Fig. 4. Effects of pH on Guanidinobutyrase.**

The activity of the enzyme was measured in the reaction mixture containing a buffer at a concentration of 50 mM. The buffers were Tris-hydrochloride, pH 7.0; Na-Bicine, pH 7.5—9.0; Na carbonate, pH 10—11. Other conditions were the same as those of the standard assay except that 0.014 and 0.03 unit of guanidinobutyrase was used for the assay with guanidinobutyrate and L-arginine, respectively. The activities toward (○), 4-guanidinobutyrate; (△), L-arginine.

**Fig. 5. Effects of Inhibitors on Guanidinobutyrase.**

The reaction rate was measured at the guanidinobutyrate concentration indicated in the presence or absence of an inhibitor: 1, none; 2, 5.0 mM \( n \)-valerate; 3, 2.5 mM 5-aminovalerate; 4, 0.05 mM thioglycolate; 5, 5.0 mM mercaptoethanolamine. V, the reaction rate in \( \mu \text{mol urea/min/mg protein} \); [GB], the concentration of 4-guanidinobutyrate in mM.
complete dissociation of the metal have been unsuccessful.

Various thiol compounds strongly inhibit the Brevibacterium enzyme. These compounds do not inhibit the Pseudomonas enzyme at 1.0 mM or below (data not shown). Inhibition by thioglycolate is about 90% at a concentration of 0.1 mM and its mode is mixed type, suggesting that this compound interacts with bound Zn\(^{2+}\). Inhibition by thiol compounds has also been observed with the enzyme preparations from the other coryneform strains which can use L-arginine\(^{5}\) and those from B. ammoniagenes, B. stasions, and B. linens, which were used in this study (data not shown).

Inhibition by lower fatty acids is of competitive type. The Pseudomonas enzyme is also inhibited competitively by lower fatty acids.\(^{13}\) n-Butyrate and n-valerate inhibit the Brevibacterium enzyme more strongly than agmatine, 5-aminovalerate, and 6-aminoacaprate. These results suggest that a carboxyl group or a negatively charged group at an end of the molecule and an adjacent short chain of methylene groups are essential for compounds to have the affinity for the active site of the enzyme. This view is supported by the fact that thioglycolate, 2-mercaptopropionate, and 3-mercaptopropionate inhibit the enzyme more strongly than 2-mercaptoethanol and 2-mercaptoethylamine.

In the arginine oxygenase pathway, 4-guanidinobutyrate is formed via 4-guanidinobutyramide. In fluorescent Pseudomonas species, many of which have the arginine oxidase pathway,\(^{3,7}\) 4-guanidinobutyrate is formed via 2-ketoarginine and 4-guanidinobutyraldehyde. In both types of organisms, 4-guanidinobutyrate is hydrolyzed by the action of guanidinobutyrase to urea and 4-aminoxbutyrate, which is degraded further by aminobutyrate aminotransferase and succinate-semialdehyde dehydrogenase. The latter two enzymes are found widely among bacteria and participate also in the degradation of putrescine. Guanidinobutyrase has a somewhat restricted distribution; among Pseudomonas strains,\(^{3,4}\) the coryneform-streptomycete group of bacteria, and Serratia marcesens (Yorifui and Tokuda, unpublished). Succinate-semialdehyde dehydrogenase of B. helvolum has been characterized in our laboratory. The enzyme has an almost the same subunit size as that of NAD\(^{+}\)-dependent enzyme of Pseudomonas. In contrast, the Zn\(^{2+}\)-guanidinobutyrase of Brevibacterium has properties rather different from the Mn\(^{2+}\)-enzyme of Pseudomonas. These facts may indicate that the dehydrogenase has evolved earlier than guanidinobutyrase, in the common ancestor of these bacteria and that the two types of guanidinobutyrase have evolved independently after divergence of each group to which each of these bacteria belongs.

While the Brevibacterium enzyme has a high binding specificity for \(\omega\)-guanidino acids, it has a weak arginine activity. The ratio of both activity of the crude enzyme is about the same as that of the purified enzyme. Both activities of the purified enzyme are lost in pararell on heating. These results indicate that the hydrolysis of L-arginine is catalyzed by guanidinobutyrase. Guanidinobutyrase of A. globiformis has also weak arginine activity (Yorifui, Shimizu, and Imada, unpublished). We have confirmed in our previous study\(^{31}\) that Pseudomonas guanidinobutyrase does not act on L-arginine, and therefore only the Pseudomonas enzyme is useful for the enzymic assay of arginine monoxygenase. When L-arginine is supplied from the cell environment, the de novo biosynthesis of L-arginine via L-ornithine is repressed in many bacteria. Under such conditions, many arginine degradation pathways of bacteria can be used for the biosynthesis of polyamines. In contrast, none of the intermediates of the arginine oxygenase pathway are useful for polyamine synthesis. Arginase and arginine deiminase were not detected in the extracts of arginine-grown cells of B. helvolum, A. globiformis, and A. simplex.\(^{5}\) These facts open the question of how these bacteria do synthesize polyamines when L-arginine is present in their environment. The arginine activity of guanidinobutyrase of coryneform bacteria suggests a possibility that the activity is involved in the polyamine synthesis.

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References