**Note**

Degradation of 2-Ketoarginine by Guanidinobutyrase in Arginine Aminotransferase Pathway of *Brevibacterium helvolum*

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Guanidinobutyrase (EC 3.5.3.7) involved in the arginine oxygenase pathway of *Brevibacterium helvolum* IFO 12073 was found to catalyze also the hydrolysis of 2-ketoarginine (2-keto-5-guanidinovalerate) to 2-ketoornithine (2-keto-5-aminovalerate) and urea, the second step of the arginine aminotransferase pathway. No other enzyme that degraded 2-ketoarginine was found in cells grown in L-arginine. The enzyme hydrolyzed L-2-ketoarginine with a relative rate of about 0.7% of that toward 4-guanidinobutyrate. The $K_m$ for 2-ketoarginine was 33 mM.

Several L-arginine degradation pathways have been found in bacteria. The arginine oxygenase pathway is distributed among Streptomyces griseus and some bacteria related phylogenetically to actinomyces, including *Arthrobacter globiformis* IFO 12173 (ATCC 8010), *Brevibacterium helvolum* IFO 12073, and *Nocardioidea simplex* IFO 12069 (*Arthrobacter simplex* ATCC 6946). On the other hand, Tsuchikura et al. have reported that *N. simplex* has the arginine aminotransferase pathway, which degrades L-arginine to 2-ketoornithine (2-keto-5-aminovalerate) via 2-ketoarginine (2-keto-5-guanidinovalerate). Although the enzyme for the first step of the pathway was identified as arginine aminotransferase, the enzyme that hydrolyzes 2-ketoarginine to 2-ketoornithine and urea has not been identified nor characterized.

We reported previously that *A. globiformis*, *B. helvolum*, and *N. simplex* had both the oxygenase and aminotransferase pathways. The third step of the oxygenase pathway is the hydrolysis of 4-guanidinobutyrate to 4-aminobutyrate and urea. This reaction is catalyzed by guanidinobutyrase (EC 3.5.3.7), which is induced by both L-arginine and 4-guanidinobutyrate. Although the enzyme of *B. helvolum* induced by L-arginine was purified to homogeneity and characterized, the activity toward L-2-ketoarginine has not been examined. This paper describes the participation of the enzyme in the aminotransferase pathway.

L-Arginine monohydrochloride was purchased from Nacalai Tesque (Kyoto). 4-Guanidinobutyrate, L-amino acid oxidase of *Crotalus adamanteus*, and thioglycolic acid were the products of Sigma (St. Louis). L-2-Ketoarginine was prepared by the enzymatic oxidation of L-arginine. Other chemicals were purchased from Nacalai Tesque. *B. helvolum* IFO 12073 (ATCC 11822) was obtained from the Institute for Fermentation Osaka. The cells were grown in a basal medium containing 4-guanidinobutyrate (0.2%) or L-arginine (0.2%) as the sole carbon and nitrogen source. The culture was grown in 1000 ml of the medium at 30°C for 29h under aeration.

The rate of 2-ketoarginine hydrolysis was measured under the same conditions as those of the guanidinobutyrase standard assay, except that 2-ketoarginine (25.0 mm) was added as the substrate. The reaction was done at 30°C for 30 min in 100 ml Tris–HCl buffer (pH 9.0) and the urea formed was measured colorimetrically. The enzyme unit and specific activity were defined as described previously. Protein in crude extracts was measured by the method of Lowry et al., and that in chromatography fractions was measured from the absorbance at 280 nm using an $E(1%)$ of 2.4, which has been measured for the guanidinobutyrase of *B. helvolum*.

The crude enzyme from the cells of *B. helvolum* grown on L-arginine was fractionated as follows. All operations were done at 0–5°C unless stated otherwise. Cells (2.0 g, wet weight) were disrupted with a Tomy UD-200 sonic disintegrator at 4–10°C in 4.0 ml of 30 mM potassium phosphate (K–PO₄) buffer (pH 8.0). The cell debris was centrifuged off, and the supernatant was dialyzed thoroughly against 20 mM K–PO₄ buffer (pH 8.0). The enzyme was put on a column (1.5 × 25 cm) of DEAE-Toyopearl 650M (Toso Corp., Tokyo) equilibrated with the dialysis buffer. The column was washed with 200 ml of the buffer, and then the enzyme was eluted with a linear gradient of 0–1.0 M KCl dissolved in 400 ml of the buffer; 4-ml fractions were collected. The active fractions (tubes 37–40) were pooled (15 ml) and mixed with 30 ml of the buffer to reduce the salt concentration. The enzyme was put on another DEAE-Toyopearl 650M column (1.0 × 25 cm). The column was washed with 100 ml of the buffer containing 0.15 M KCl, and then the enzyme was eluted with a linear gradient of 0.15–0.4 M KCl dissolved in 200 ml of the buffer; 2-ml fractions were collected. The active fractions (tubes 35–42) were pooled (16 ml) and concentrated to 1.2 ml by ultrafiltration. The enzyme was then put on a column (1.0 × 100 cm) of Toyopearl HW-55F (Toso Corp., Tokyo) equilibrated with 20 mM K–PO₄ buffer (pH 8.0) containing 0.2 M KCl. The enzyme was eluted with the equilibration buffer; 1.0-ml fractions were collected. The active fractions (tubes 47–52) were pooled (6.0 ml). The enzyme was mixed with 6.0 ml of 50% (w/v) glycerol and stored at −20°C. The enzyme induced by 4-guanidinobutyrate was purified under conditions similar to those described above. The purification of the enzyme was summarized in the Table 1.

### Table 1. Purification of Guanidinobutyrases with Activities toward 2-Ketoarginine

<table>
<thead>
<tr>
<th>Step</th>
<th>Protein (mg)</th>
<th>Total units</th>
<th>Sp. act. (units/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude extract</td>
<td>85&lt;sup&gt;a&lt;/sup&gt;</td>
<td>85</td>
<td>1.0</td>
</tr>
<tr>
<td>1st DEAE-Toyopearl</td>
<td>20&lt;sup&gt;b&lt;/sup&gt;</td>
<td>59</td>
<td>3.0</td>
</tr>
<tr>
<td>2nd DEAE-Toyopearl</td>
<td>1.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>17</td>
<td>17</td>
</tr>
<tr>
<td>Toyopearl HW-55F</td>
<td>2.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>25</td>
<td>13</td>
</tr>
</tbody>
</table>

* Measured by the method of Lowry et al.
* Measured from A₂₈₀ using an E₁% valeur of 2.4 of the homogeneous guanidinobutyrase.
* Measured under the standard guanidinobutyrase assay conditions.

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enzymes is summarized in Table I, in which the total units were measured with 4-guanidinobutyrate as the substrate. The values in Table I-A for protein and total units after the third step may be lower than the true values, being due to some error in their measurement. The specific activity of the homogenous enzyme was reported to be 83.8 units/mg.

The rate of the hydrolysis of 2-ketoarginine with the crude extract of the cells grown on l-arginine was about 0.7% of the rate of 4-guanidinobutyrate hydrolysis, and this was very close to that obtained with the extract from the cells grown on 4-guanidinobutyrate (data not shown). When the extract from the cells grown on l-arginine was chromatographed on DEAE-Toyopearl as described above, the activity toward 2-ketoarginine was eluted as a single peak and was almost parallel with that toward 4-guanidinobutyrate (Fig. 1A). This chromatogram was similar to that obtained with the enzyme induced by 4-guanidinobutyrate (Fig. 1C). On Toyopearl HW-55F chromatography of the 2nd DEAE-Toyopearl fraction of the enzyme induced by l-arginine, the fractions that showed guanidinobutyrate activities also showed the activities toward 2-ketoarginine (Fig. 1B). This result was similar to that obtained with the enzyme induced by 4-guanidinobutyrate (Fig. 1D). The relative activities of all the active fractions described above toward 2-ketoarginine, compared with those toward 4-guanidinobutyrate, were close to 0.7%.

The substrate specificity of the partially purified enzyme induced by l-arginine was very similar to that of the enzyme induced by 4-guanidinobutyrate (Table II). The activity of the enzyme induced by l-arginine toward 2-ketoarginine was about 0.7% of that toward 4-guanidinobutyrate, and this was very close to the value obtained with the enzyme induced by 4-guanidinobutyrate. The action of the enzyme induced by l-arginine on some other guanidino compounds was reported previously. The following results were obtained with the enzyme induced by l-arginine. The optimum pH for the hydrolysis of 2-ketoarginine was 9.0, which was close to that of the guanidinobutyrase reaction. The Michaelis constant (K_m) for 2-ketoarginine was 33 mM, which was measured at the substrate concentrations of 5.0–30.0 mM; the value was higher, by one order, than the value (2.9 mM) for 4-guanidinobutyrate.

Thiglycolate is a potent inhibitor of guanidinobutyrase of B. helvolum. The effect of the inhibitor tested at various concentrations on the enzyme induced by l-arginine (Fig. 2A) was very close to that on the enzyme induced by 4-guanidinobutyrate (Fig. 2B). The reactions with 2-ketoarginine and 4-guanidinobutyrate were equally affected.

These results indicate that guanidinobutyrase catalyzes the hydrolysis of 2-ketoarginine in the arginine aminotransferase pathway (the second step), in addition to the hydrolysis of 4-guanidinobutyrate in the arginine oxidase pathway (the third step). This enzyme has also a low activity toward l-arginine, and the metabolic role of this activity was discussed in our previous paper.

References