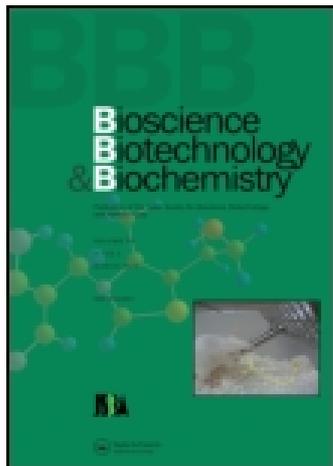


This article was downloaded by: [Chulalongkorn University]

On: 27 December 2014, At: 10:03

Publisher: Taylor & Francis

Informa Ltd Registered in England and Wales Registered Number: 1072954 Registered office: Mortimer House, 37-41 Mortimer Street, London W1T 3JH, UK



## Bioscience, Biotechnology, and Biochemistry

Publication details, including instructions for authors and subscription information:

<http://www.tandfonline.com/loi/tbbb20>

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

Takamitsu Yorifuji<sup>a</sup>, Mitsuoki Kaneoke<sup>a</sup>, Takao Okazaki<sup>a</sup> & Eiichi Shimizu<sup>a</sup>

<sup>a</sup> Department of Bioscience and Biotechnology, Shinshu University, Minamiminowa, Nagano 399-45, Japan

Published online: 12 Jun 2014.

To cite this article: Takamitsu Yorifuji, Mitsuoki Kaneoke, Takao Okazaki & Eiichi Shimizu (1995) Degradation of 2-Ketoarginine by Guanidinobutyrase in Arginine Aminotransferase Pathway of *Brevibacterium helvolum*, *Bioscience, Biotechnology, and Biochemistry*, 59:3, 512-513, DOI: [10.1271/bbb.59.512](https://doi.org/10.1271/bbb.59.512)

To link to this article: <http://dx.doi.org/10.1271/bbb.59.512>

PLEASE SCROLL DOWN FOR ARTICLE

Taylor & Francis makes every effort to ensure the accuracy of all the information (the "Content") contained in the publications on our platform. However, Taylor & Francis, our agents, and our licensors make no representations or warranties whatsoever as to the accuracy, completeness, or suitability for any purpose of the Content. Any opinions and views expressed in this publication are the opinions and views of the authors, and are not the views of or endorsed by Taylor & Francis. The accuracy of the Content should not be relied upon and should be independently verified with primary sources of information. Taylor and Francis shall not be liable for any losses, actions, claims, proceedings, demands, costs, expenses, damages, and other liabilities whatsoever or howsoever caused arising directly or indirectly in connection with, in relation to or arising out of the use of the Content.

This article may be used for research, teaching, and private study purposes. Any substantial or systematic reproduction, redistribution, reselling, loan, sub-licensing, systematic supply, or distribution in any form to anyone is expressly forbidden. Terms & Conditions of access and use can be found at <http://www.tandfonline.com/page/terms-and-conditions>

## Note

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

Takamitsu YORIFUJI, Mitsuoki KANEOKI, Takao OKAZAKI, and Eiichi SHIMIZU

Department of Bioscience and Biotechnology, Shinshu University, Minamiminowa, Nagano 399–45, Japan

Received July 4, 1994

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 on L-arginine. The enzyme hydrolyzed 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.<sup>1)</sup> The arginine oxygenase pathway is distributed among *Streptomyces griseus*<sup>2)</sup> and some bacteria related phylogenetically to actinomycetes, including *Arthrobacter globiformis* IFO 12137 (ATCC 8010), *Brevibacterium helvolum* IFO 12073, and *Nocardiooides simplex* IFO 12069 (*Arthrobacter simplex* ATCC 6946).<sup>3–5)</sup> On the other hand, Tochikura *et al.*<sup>6)</sup> 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*,<sup>4)</sup> *B. helvolum*,<sup>4)</sup> and *N. simplex*<sup>5)</sup> 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),<sup>2)</sup> which is induced by both L-arginine and 4-guanidinobutyrate.<sup>4,5)</sup> Although the enzyme of *B. helvolum* induced by L-arginine was purified to homogeneity and characterized,<sup>7)</sup> the activity toward 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). 2-Ketoarginine was prepared by the enzymatic oxidation of L-arginine.<sup>8)</sup> 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<sup>4)</sup> containing 4-guanidinobutyrate (0.2%) or L-arginine (0.2%) added as the sole carbon and nitrogen source. The culture was grown in 1000 ml of the medium at 30°C for 29 h under aeration.

The rate of 2-ketoarginine hydrolysis was measured under the same conditions as those of the guanidinobutyrase standard assay,<sup>7)</sup> except that 2-ketoarginine (25.0 mM) was added as the substrate. The reaction was done at 30°C for 30 min in 100 mM Tris-HCl buffer (pH 9.0) and the urea formed was measured colorimetrically.<sup>9)</sup> The enzyme unit and specific activity were defined as described previously.<sup>7)</sup> Protein in crude extracts was measured by the method of Lowry *et al.*,<sup>10)</sup> 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 guani-

dinobutyrase of *B. helvolum*.<sup>7)</sup>

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-PO<sub>4</sub>) buffer (pH 8.0). The cell debris was centrifuged off, and the supernatant was dialyzed thoroughly against 20 mM K-PO<sub>4</sub> buffer (pH 8.0). The enzyme was put on a column (1.5 × 25 cm) of DEAE-Toyopearl 650M (Tosoh 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 (Tosoh Corp., Tokyo) equilibrated with 20 mM K-PO<sub>4</sub> 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

**Table I.** Purification of Guanidinobutyrases with Activities toward 2-Ketoarginine

A. Extract of cells grown on L-arginine.

| Step               | Protein (mg)     | Total units <sup>c</sup> | Sp. act. (units/mg) |
|--------------------|------------------|--------------------------|---------------------|
| Crude extract      | 85 <sup>a</sup>  | 85                       | 1.0                 |
| 1st DEAE-Toyopearl | 20 <sup>b</sup>  | 59                       | 3.0                 |
| 2nd DEAE-Toyopearl | 1.0 <sup>b</sup> | 17                       | 17                  |
| Toyopearl HW-55F   | 2.0 <sup>b</sup> | 25                       | 13                  |

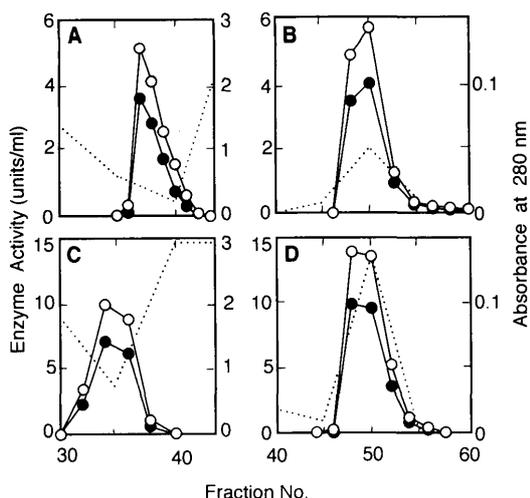
B. Extract of cells grown on 4-guanidinobutyrate.

| Step               | Protein (mg)     | Total units <sup>c</sup> | Sp. act. (units/mg) |
|--------------------|------------------|--------------------------|---------------------|
| Crude extract      | 140 <sup>a</sup> | 273                      | 2.0                 |
| 1st DEAE-Toyopearl | 125 <sup>b</sup> | 254                      | 2.0                 |
| 2nd DEAE-Toyopearl | 11 <sup>b</sup>  | 180                      | 16                  |
| Toyopearl HW-55F   | 3.2 <sup>b</sup> | 110                      | 34                  |

<sup>a</sup> Measured by the method of Lowry *et al.*<sup>10)</sup>

<sup>b</sup> Measured from  $A_{280}$  using an  $E(1\%)$  value of 2.4 of the homogeneous guanidinobutyrase.<sup>7)</sup>

<sup>c</sup> Measured under the standard guanidinobutyrase assay conditions.<sup>7)</sup>



**Fig. 1.** Elution Profiles of Enzyme Activities toward 2-Ketoarginine and 4-Guanidinobutyrate from DEAE-Toyopearl and Toyopearl Columns.

The elution profiles of: A, the extract from the cells grown on L-arginine on DEAE-Toyopearl 650 M; B, the 2nd DEAE-Toyopearl fraction of the enzyme induced by L-arginine on Toyopearl HW-55F; C, the extract from the cells grown on 4-guanidinobutyrate on DEAE-Toyopearl 650 M; D, the 2nd DEAE-Toyopearl fraction of the enzyme induced by 4-guanidinobutyrate on Toyopearl HW-55F. Solid line, the enzyme activity toward 4-guanidinobutyrate (○) and the activity toward 2-ketoarginine multiplied by a factor of 100 (●). Dotted line, absorbance at 280 nm. The other experimental conditions are given in the text.

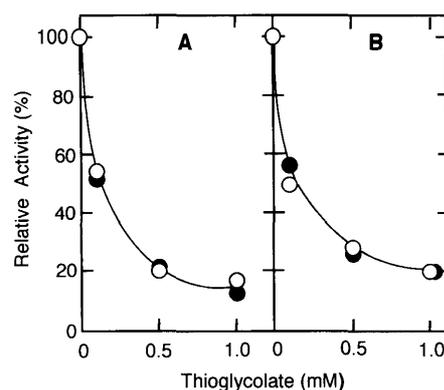
**Table II.** Substrate Specificity of Guanidinobutyrase with Activities toward 2-Ketoarginine

| Compound            | Relative activity (%) |                     |
|---------------------|-----------------------|---------------------|
|                     | Inducer               |                     |
|                     | L-Arginine            | 4-Guanidinobutyrate |
| 4-Guanidinobutyrate | 100                   | 100                 |
| L-Arginine          | 0.75                  | 0.71                |
| 2-Ketoarginine      | 0.71                  | 0.68                |

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 homogeneous enzyme was reported to be 83.8 units/mg.<sup>7)</sup>

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 guanidinobutyrase 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



**Fig. 2.** Effects of Thioglycolate on Enzymic Hydrolyses of 2-Ketoarginine and 4-Guanidinobutyrate.

The partially purified enzyme induced by L-arginine (A) or that induced by 4-guanidinobutyrate (B) was added to the reaction mixture containing thioglycolate at the concentration indicated. Enzyme activity was measured with 2-ketoarginine (●) or 4-guanidinobutyrate (○) as the substrate. The other reaction conditions were the same as those of the standard enzyme assay.

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.<sup>7)</sup> 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.<sup>7)</sup> 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.<sup>7)</sup>

Thioglycolate is a potent inhibitor of guanidinobutyrase of *B. helvolum*.<sup>7)</sup> 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 oxygenase pathway (the third step). This enzyme has also a low activity toward L-arginine,<sup>7)</sup> and the metabolic role of this activity was discussed in our previous paper.<sup>7)</sup>

## References

- 1) R. Cunin, N. Glansdorff, A. Piérard, and V. Stalon, *Microbiol. Rev.*, **50**, 314–352 (1986).
- 2) N. van Thoai and A. Olomucki, *Biochim. Biophys. Acta*, **59**, 533–544 (1962).
- 3) T. Yorifuji, K. Hirabayashi, T. Nagashima, N. Inagaki, E. Shimizu, K. Imada, T. Katsumi, and S. Sawamura, *Agric. Biol. Chem.*, **53**, 1103–1110 (1989).
- 4) M. Kaneoke, K. Shiota, M. Kusunose, E. Shimizu, and T. Yorifuji, *Biosci. Biotech. Biochem.*, **57**, 814–820 (1993).
- 5) M. Kaneoke, E. Shimizu, and T. Yorifuji, *Biosci. Biotech. Biochem.*, **58**, 244–249 (1994).
- 6) T. Tochikura, K. Sugiyama, M. Bunno, T. Matsubara, and T. Tachiki, *Agric. Biol. Chem.*, **44**, 1773–1778 (1980).
- 7) T. Yorifuji, E. Shimizu, H. Hirata, K. Imada, T. Katsumi, and S. Sawamura, *Biosci. Biotech. Biochem.*, **56**, 773–777 (1992).
- 8) A. Meister, *J. Biol. Chem.*, **76**, 309–317 (1952).
- 9) L. M. Prescott and M. E. Jones, *Anal. Biochem.*, **32**, 408–419 (1969).
- 10) O. H. Lowry, N. J. Rosebrough, A. L. Farr, and R. J. Randall, *J. Biol. Chem.*, **193**, 265–275 (1951).