Bull. Inst. Chem. Res., Kyoto Univ., Vol. 65, No. 3, 1987

NOTE

Microbial Production of D-Valine from Racemic α-Aminoisovaleronitrile

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Received May 16, 1987

KEY WORDS: D-Valine production/ DL-α-Aminoisovaleronitrile/ Stereospecific microbial production/

Stereoselectivity is one of the most salient characteristies of enzymes. Thus, enzymes and microbial cells as enzyme bags have been widely used as effective biocatalysts for the industrial prodiction of a variety of optically active compounds such as L- and D-amino acids. Various optically active amino acids now are produced microbially and enzymatically.[1-5]

Aminonitrile compounds are hydrolyzed chemically to their corresponding racemic amino acids.

Fukuda et al. studied microbial hydrolysis of DL-aminonitriles, and found that racemic amino acids were produced from racemic aminonitriles by incubation with the cell suspensions of a strain of *Corynebacterium* sp. HR.[6] Asano et al. reported that the nitrilase activity of *Arthrobactor* sp. is effected by combination of nitrile hydratase and amidase,[7] but a single enzyme, nitrilase, also catalyzes hydrolysis of nitrile compounds to the corresponding carboxylic acids.[8] Recently, Arnand et al. isolated a D-amidase-less mutant of *Brevibacteruim* sp. which produces L-amino acids and D-amino amides from DL-aminonitriles.[9]

We describe here the stereospecific production of D-valine from DL-α-aminoisovaleronitrile by means of mycelia of *Fusarium oxysporum* IFO 5942. The enzyme(s) system participating to the prodiction of D-valine is also discussed.

DL-α-aminoisovaleronitrile, DL-, D- and L-valineamide were kindly supplied from Research center, Daicel Chemical Industries, Ltd., Hyogo, Japan. The other chemicals were analytical grade reagents. *Fusarium oxysporum* was grown aerobically in the medium (pH 6.0) containing 1% polypeptone, 0.5% meat extract, 0.5% yeast extract, and 0.5% NaCl at 30°C for 18 hr. The mycelia were collected by centrifugation and washed with 0.1 M potassium phosphate buffer (pH 7.2).

Mycelia of the *Fusarium* (50 mg, wet weight) were incubated with the standard reaction mixture (1 ml) containing 100 μmol of DL-α-aminoisovaleronitrile and 100 μmol of potassium phosphate buffer (pH 7.2). D-Valine formed was determined by enantioselective high per-

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formance liquid chromatography with the authentic D-valine (retention time, 20.0 min) on Daicel Chiralpak WH column (4.6×250 mm) (mobile phase, 0.25 mM copper sulfate; flow rate, 1.0 ml per min; at 50°C). The remaining DL-α-aminoisovaleronitrile and DL-valineamide were determined spectrophotometrically after extraction from the silica gel 60 F254 plates (Merck) with a solvent, *n*-butanol-acetic acid-H_2O (12:3:5, v/v/v) according to the procedure of Watanabe et al.[10]

As the incubation proceeded, the concentration of DL-α-aminoisovaleronitrile decreased and that of D-valine increasde as shown in Fig. 1. After incubation for 10 hr, 30 μmol of D-valine was produced, and 58 μmol of DL-α-aminoisovaleronitrile was remained. None of L-valine and DL-valineamide were formed during the reaction. When the reaction mixture was incubated larger, and with more than 150 mg (wet weight) of the mycelia, L-valine was produced gradually to accumulate about 3.6 μmol, and 35.6 μmol of the enantiomer was formed.

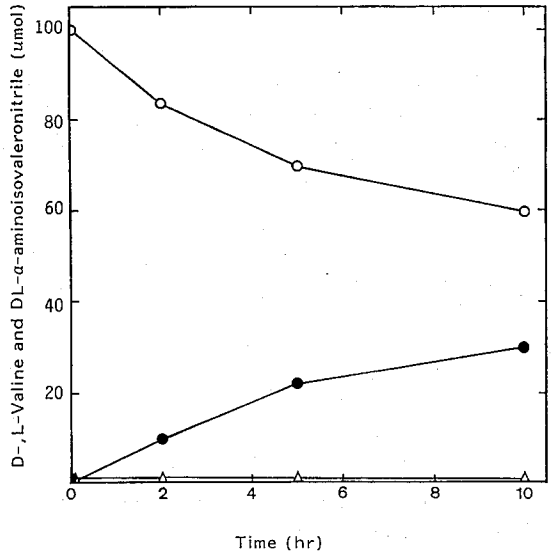

Fig. 1. Formation of D-Valine from DL-α-Aminoisovaleronitrile. The reaction mixture (1 ml) contained 50 mg (wet weight) of the mycelia, 100 μmol of DL-α-aminoisovaleronitrile, and 100 μmol of potassium phosphate buffer (pH 7.2). Incubation was carried out at 30°C. (●), D-valine; (▲), L-valine; (○), DL-α-aminoisovaleronitrile.

When 100 μmol of DL-valineamide was incubated for 10 hr with the mycelia in the standard reaction mixture, nearly 86% of initial amount of DL-valineamide was converted to DL-valine (D-valine, 38.0 μmol; L-valine, 48.0 μmol). Both of D- and L-isomers of valineamide probably were hydrolyzed by the amidase as reproted previously.[7]

After incubation with 50 mg of the mycelia for 10 hr, the remaining DL-α-aminoisovaleronitrile was separated by preparative thin layer chromatography and hydrolyzed with 6N HCl as described previously.[10] The hydrolysate containing valine was analyzed by the enantioselective high performance liquid chromatography. L-α-aminoisovaleronitrile remained

Table I. Analysis of isomers of remaining DL-α-aminoisovaleronitrile after D-valine production by *Fusarium oxysporum* IFO 5942

Incubation time (hr)	Percentage of isomers of remaining DL-α-aminoisovaleronitrile (%)	
	D	L
0	48	52
10	24	76

The reaction conditions were identical to that of in Fig. 1. Remaining DL-α-aminoisovaleronitrile separated by thin layer chromatography was analyzed by enantioselective high performance liquid chromatography after the 6N HCl hydrolysis as described in the text. Percentage of isomers of remaining DL-α-aminoisovaleronitrile is expressed as valine converted by the 6N HCl hydrolysis.

predominantly (Table I). This suggests that D-α-aminoisovaleronitrile is probably hydrolyzed by a D-sterospecific aminonitrilase to form D-valine, although the L-isomer of substrate also is hydrolyzed more slowly enzymatically or non-enzymatically.

D-Valine was produced with the mycelia grown in the medum (pH 7.2) containing 1% propionitrile, 0.5% glycerol, 0.2% K_2HPO_4, 0.2% KH_2PO_4, 0.1% NaCl, and 0.02% yeast extract at 30°C for 72 hr,[6] as well as with the mycelia grown in the medium containing polypeptone, meat extract, and yeast extract as described above. Therefore, the D-aminonitrilase is probably formed constitutively.

(Acknowledgment: We wish to thank Professor Ken'ichi Okamoto, Department of Food and Nutrition, Okayama Prefectural Junior College for his helpful advice.)

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