α -Aminoisobutyrate Decomposing Enzyme from *Penicillium oxalicum*

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Enzymatic decomposition of various α -dialkyl- α -amino acids to the respective ketones consists of the decarboxylation and the transamination toward the second substrate, α -keto acid, and is also called decarboxylation-dependent transamination.¹⁾ α -Aminoisobutyrate (AIB) decomposing enzyme, which catalyzes this reaction, has been found and purified from several species of bacteria, *Pseudomonas*.^{1~3)} The present paper describes that fungi, *Penicillium*, are also able to catalyze this reaction.

Fungi were incubated on AIB medium agar containing 10% sucrose, 0.2% AIB, 0.1% KH_2PO_4 , 0.05% $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.01% $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ and 2% agar. Results in Table I show that AIB supported good growth of many fungi as the sole nitrogen source. The same experiments were done with other two α -dialkyl- α -amino acids, *i.e.*, α -methyl-DL-serine and 1-aminocyclopropane-1-carboxylic acid. Both amino acids were assimilated by many fungi. Especially, the growth pattern of every fungus on α -methyl-DL-serine medium was similar to that on AIB, implying that an identical enzyme may participate in the metabolism of these two amino acids. Thus, from Table I, it was suggested that various microorganisms might be capable of metabolizing these α -dialkyl- α -amino acids.

In order to compare fungus enzyme with *Pseudomonas* enzyme, it was partially purified from *Penicillium oxalicum*. This fungus was statically cultured at 27°C for 3 days in the AIB medium containing 3% sucrose, 0.2% AIB, 0.1% K_2HPO_4 , 0.05% $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.05% KCl, 0.001% $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ and 0.01% dried yeast extract. As the culture medium became acidic after the incubation period, mycelia were further shaken in 0.1 M potassium phosphate buffer, pH 7.5, containing 0.2% AIB and 3% sucrose at 27°C for one day. Mycelia obtained, 39 g, were ground with sea sand in 0.1 M potassium phosphate buffer, pH 7.5, containing 10^{-5} M pyridoxal 5'-phosphate, which was used as solvent throughout all steps of purification. Crude extract obtained by centrifugation had 1333 mg of protein and 42.7 units of AIB decomposing enzyme which were assayed as described previously.⁴⁾ Solid ammonium

TABLE I. GROWTH ON A MEDIA CONTAINING α -DIALKYL- α -AMINO ACID AS THE SOLE NITROGEN SOURCE

Each fungus was incubated at 27°C for 7 or 20 days and its growth was represented by symbols, *i.e.*, luxuriant (++) or distinguishable (+) or undistinguishable (−) from the control growth in a medium in which no nitrogen source was added.

Species	AHU No.	Growth on		
		AIB	ACPC ^a	MS ^b
<i>Penicillium</i>				
<i>crustosum</i>	8002	++	++	++
<i>funiculosum</i>	8014	—	++	—
<i>roqueforti</i>	8033	+	+	—
<i>notatum</i>	8035	++	+	++
<i>oxalicum</i>	8046	+	+	++
<i>spinulosum</i>	8068	++	++	++
<i>lilacinum</i>	8021	++	++	++
<i>islandicum</i>	8086	—	++	—
<i>javanicum</i>	8028	—	++	—
<i>digitatum</i>	8004	+	+	±
<i>chrysogenum</i>	8117	++	+	+
<i>italicum</i>	8018	+	+	+
<i>flavo-glaucum</i>	8013	++	+	++
<i>camemberti</i>	8113	+	+	±
<i>expansum</i>	8011	++	+	++

^a 1-Aminocyclopropane-1-carboxylic acid.

^b α -Methyl-DL-serine.

sulfate was added to the crude extract to give a final concentration of 20% saturation. The mixture was centrifuged and to the supernatant, ammonium sulfate was added up to 50% saturation. Resulting precipitate was dissolved in a small volume of the buffer and applied to gel filtration with a Bio-Gel A-0.5 m column (3 × 55 cm). The active fraction obtained was further fractionated by hydrophobic chromatography⁵⁾ with a aminohexyl-Sepharose column (2 × 7 cm). The enzyme preparation was eluted from the column with a linear gradient of KCl concentration, 0.3 to 0.45 M. This preparation had 13.1 mg of protein and 11.7 units of the enzyme and consisted of a single active protein and much impurity as known from results of disc electrophoresis.⁶⁾

Relative rates of decarboxylation of α -dialkyl- α -amino acids were measured manometrically with the partially purified fungus enzyme and compared with those of *Pseudomonas* enzyme (Table II). Three amino acids were more active than AIB, and α -methyl-DL-serine was the best substrate. In addition, relative activities of 2-methyl-D-serine and hydroxymethylserine were higher than those of *Pseudomonas* enzyme. Therefore, the substrates containing hydroxymethyl group were more strongly decarboxylated by the fungus enzyme, being compared with the bacterial enzyme.

TABLE II. RELATIVE ACTIVITIES OF VARIOUS
 α -DIALKYL- α -AMINO ACIDS OR
 AMINO ACCEPTORS

Enzyme was incubated in 0.1 M potassium phosphate buffer containing 0.1 mM pyridoxal 5'-phosphate, 75 mM amino acid and 10 mM α -keto acid. For amino acid specificity, liberation of carbon dioxide was measured at pH 7.0 with a reaction mixture containing pyruvate as amino acceptor and for amino acceptor specificity, formation of acetone from AIB was done at pH 7.5. Values in parentheses are relative activities of *Pseudomonas* enzyme.^{3,7,8)}

Amino acid	Relative activity
AIB	100 (100)
α -Methyl-DL- α -aminovaleric acid	164 (38)
α -Methyl-DL-valine	36 (38)
α -Ethyl- α -aminobutyric acid	18 (33)
α -Methyl-D- α -aminobutyric acid	24 (18)
α -Methyl-L- α -aminobutyric acid	141 (54)
α -Methyl-DL-serine	229 (62)
α -Methyl-D-serine ^a	51 (14)
1-Aminocyclopropane-1-carboxylic acid	0 (0)
Hydroxymethylserine	60 (8)
Amino acceptor	
Glyoxylate	7 (10)
Pyruvate	123 (96)
α -Ketobutyrate	100 (100)
α -Ketovalerate	10 (13)
α -Ketocaproate	4 (1)
α -Ketoglutarate	0 (0)
Hydroxypyruvate	39 (7)

^a D-Isomer was prepared from α -methyl-DL-serine by *Pseudomonas* enzyme.⁷⁾

The same goes with hydroxypyruvate as amino acceptor (Table II).

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