Monoamine Transamination Catalyzed by ω -Amino Acid: Pyruvate Aminotransferase of *Pseudomonas* sp. F-126

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ω -Amino acid: pyruvate aminotransferase of *Pseudomonas* sp. F-126 catalyzes stoichiometrically a transamination between various amines and pyruvate. Most of alkyl and aromatic monoamines served as an amino donor. The enzyme activity was affected by carbon number of straight-chain alkylmonoamines with a maximum activity at 5-carbon unit, *n*-amylamine. Michaelis constants for *n*-butylamine and pyruvate were calculated to be 66.6 mM and 5.5 mM respectively. The enzyme was active in the alkaline range with a maximum at pH 10.5~11.0, though not any activity was observed at the pH below 8.0. The optimum temperature for the reaction was at 60°C.

The occurrence of various ω -amino acid aminotransferase has been demonstrated in animal and plant tissues, and in microorganisms,^{1,2)} and some of them have been purified and characterized.^{3~8)} We reported the purification and crystallization of ω -amino acid: pyruvate aminotransferase of *Pseudomonas* sp. F-126 to elucidate the enzymologic properties.^{9,10)} The enzyme has a molecular weight of 172,000. The enzyme was characterized by its low substrate specificity for ω -amino acid. Most of ω -amino acids are more or less transaminated by the enzyme with pyruvate, an exclusive amino acceptor. Among them 2-aminoethane sulfinate (hypotaurine), 3-amino-propane sulfonate, β -alanine, γ -aminobutyrate, 8-aminooctanoate, and DL- β -amino-*n*-butyrate were good amino donors, while α,ω -diamino-carboxylic acids *i.e.* L-ornithine, D- and L-lysine were inert. The enzyme also showed to be active to such amines as ethylamine, ethanolamine, *n*-butylamine, histamine, and putrescine.¹⁰⁾ In spite of the similar reactivity of amino group of ω -amino acids and amines, it was generally accepted that transamination of these compounds was catalyzed by a separate enzyme. Present communication describes further investigations on the transamination between monoamines and pyruvate catalyzed by ω -amino acid: pyruvate aminotransferase.

MATERIALS AND METHODS

Chemicals. β -Alanine, salicylaldehyde, *n*-butylaldehyde and amines were purchased from Nakarai Chemicals, Kyoto; sodium pyruvate from Merck. *o*-Aminobenzaldehyde was synthesized by reduction of *o*-nitrobenzaldehyde according to the method of Smith and Optie.¹¹⁾ Amine oxidase from *Aspergillus niger* was kindly provided by Dr. Hideaki Yamada, Kyoto University.

Enzyme preparation. ω -Amino acid: pyruvate aminotransferase of *Pseudomonas* sp. F-126 was purified to homogeneity and crystallized by the method described previously with a modification. Cultivation of the microorganism, extraction of the enzyme, polyethyleneimine treatment and DEAE-cellulose column chromatography were carried out as described before.⁹⁾ To the enzyme fraction through DEAE-cellulose column was added ammonium sulfate (30% saturation). After the precipitate was removed by centrifugation, ammonium sulfate was added to the supernatant until the solution became a slightly turbid, and then a small amount of the crystal of the enzyme was added. The enzyme was crystallized at 4°C for 12 hr. The crystals were collected by centrifugation and dissolved in 0.02 M potassium phosphate buffer (pH 7.2), and then applied on a column of Sephacryl S-200 (Pharmacia) equilibrated with the same buffer. The enzyme fraction showing a single band on a Disc-gel electrophoresis were pooled and crystallized by addition of ammonium sulfate. The enzyme activity was recovered in a yield of 50~60%.

Enzyme assay. The reaction mixture contained 50 μ mol of amines, 20 μ mol of sodium pyruvate,

1 μ mol of pyridoxal 5'-phosphate (pyridoxal-P), 100 μ mol of K_2HPO_4 -KOH buffer (pH 10.0) and enzyme in a final volume of 1.0 ml. In a blank amine was replaced by water. After incubation for 20 min the reaction was terminated by addition of 0.1 ml of 25% trichloroacetic acid, followed by centrifugation. The enzyme activity was assayed by determining alanine (method A) or aldehyde (method B) of the supernatant with an amino acid analyzer (Nihon Denshi JLC-6AH) and *o*-aminobenzaldehyde, respectively as described previously.¹⁰⁾

Other assay. The amount of *n*-butylamine was determined by amine oxidase. The amine oxidase reaction was performed according to Yamada *et al.*¹²⁾ The enzyme activity was assayed by determining *n*-butylaldehyde with *o*-aminobenzaldehyde. Pyruvate was determined by a salicylaldehyde method.¹³⁾ Protein concentration of the enzyme was derived from the absorbance at 280 nm. The absorbance coefficient ($A_{1\%}^{1\text{cm}}, 280\text{nm} = 9.84$) was obtained by absorbance and dry weight determinations.

RESULTS

Amine transamination by ω -amino acid: pyruvate aminotransferase

Although the enzyme was crystallized and homogenous on the criteria of Disc-gel electrophoresis and ultracentrifugation, a possibility was remained that the amine transamination was due to an amine transaminase contaminated in the enzyme preparation. Experiments were performed to demonstrate that ω -amino acid: pyruvate aminotransferase catalyzed amine transamination. The thermal stability of the activity of amine transamination was examined in the presence or the absence of pyridoxal-P (Fig. 1 A). The enzyme activity was stable up to 65°C, and inactivated over 70°C. By incubation with pyridoxal-P at 40~60°C, an increase in the enzyme activity was observed to some extent. The same results were obtained for ω -amino acid transamination.¹⁰⁾ The activity of amine aminotransferase was stable at pH 8.0~10.0 (Fig. 1 B), which was also observed for that of ω -amino acid aminotransferase. We previously reported that the activity of ω -amino acid aminotransferase was markedly inhibited by typical inhibitors for pyridoxal-P enzymes such as phenylhydrazine,

FIG. 1. Effect of Temperature (A) and pH (B) on Stability of Amine Transamination.

n-Butylamine was used as an amino donor. (A): The enzyme was incubated at the indicated temperatures for 10 min (pH 8.0) and then the activity was determined by assay method B in the presence (○—○) or the absence (●—●) of pyridoxal-P. (B): After the enzyme was incubated in the following buffers at 37°C for 10 min, the activity was measured by assay method B: 0.1 M CH_3COOH - CH_3COONa (pH 5.0~5.5), 0.1 M K_2HPO_4 - KH_2PO_4 (pH 6.0~8.0), 0.1 M Tris-HCl (pH 8.0~8.5), 0.1 M glycine-KCl-KOH (pH 9.0~11.0) and 0.1 M K_2HPO_4 -KOH (pH 11.0~12.0).

3-methyl-2-benzothiazolone hydrazone hydrochloride, aminoxyacetate, hydroxylamine and D-cycloserine, but not affected with sulfhydryl reagents ($HgCl_2$, *p*-chloromercuribenzoate, N-ethylmaleimide, moniodoacetate and 5,5'-dithio-bis(2-nitrobenzoic acid)) and chelating agents (ethylenediaminetetraacetate, diethylthiocarbamate, tiron, 8-oxyquinoline, *o*-phenanthroline and α, α' -dipyridyl). The case was also true for the activity of amine aminotransferase. Both activities of ω -amino acid and amine aminotransferase were induced by addition of β -alanine in the cultivation medium. Not any activities, however, of either enzymes could be detected by the replacement of β -alanine with *n*-butylamine. All these results indicate that transaminations of ω -amino acid and amine are catalyzed by a single enzyme.

Stoichiometry

The stoichiometry of the transamination between *n*-butylamine and pyruvate was ex-

aminated. After the standard reaction mixture was incubated for 30 and 60 min, all of the reactants and the products were determined. The reaction mixture to which trichloroacetate was added before initiation of the reaction was used as a control. This enzymatic transamination was found to proceed stoichiometrically, as shown in Table I.

TABLE I. STOICHIOMETRY OF THE REACTION

After the enzyme reaction was performed at 37°C for the indicated time, amounts of the reactant and the product were determined as described in text.

Incubation time (min)	<i>n</i> -Butylamine (μmoles)	Pyruvate (μmoles)	<i>n</i> -Butylaldehyde (μmoles)	L-α-Alanine (μmoles)
0	11.9	12.0	0	0
30	9.3	9.1	2.6	2.8
60	7.5	7.4	4.3	4.6

pH and temperature optima

The pH dependence of transamination between *n*-butylamine and pyruvate was examined in various buffers. The enzyme was active in the alkaline region with a maximum at 10.5~11.0 (Fig. 2 A). Although the transamination of ω -amino acid was observed at the pH between 5.0~12.0, the activity of amine aminotransferase could not be detected

FIG. 2. Optimum pH (A) and Temperature (B) of the Amine Transamination.

The enzyme activity was determined by assay method B at various pH and temperatures using *n*-butylamine as an amino donor. The following buffers were used: ○—○, 0.1 M K₂HPO₄-KH₂PO₄ (pH 8.0~8.5), ●—●, glycine-KCl-KOH (pH 9.0~12.0), ▲—▲, 0.1 M K₂HPO₄-KOH (pH 9.5~12.0).

at the pH below 8.0, indicating that a non-protonated form of amino group of amines participates in the reaction.

The aminotransferase reaction was performed at various temperatures using *n*-butylamine as an amino donor (Fig. 2 B). The maximum activity was obtained at 60°C. The reaction velocity increased with increasing the temperature from 30°C to 60°C, but over 70°C it decreased rapidly.

Substrate specificity

The ability of the enzyme to catalyze transamination between various amines and pyruvate is presented in Table II. The enzyme activity is represented by a relative activity to

TABLE II. AMINO DONOR SPECIFICITY

The enzyme activity was assayed by determining L-α-alanine with an amino acid analyzer (Method A).

Amino donor	Rel. act.
β-Alanine	100
Methylamine	0
Ethylamine	30
Ethanolamine	5
<i>n</i> -Propylamine	35
Isopropylamine	40
<i>n</i> -Butylamine	60
Isobutylamine	50
<i>sec</i> -Butylamine	30
<i>t</i> -Butylamine	0
<i>n</i> -Amylamine	80
Isoamylamine	30
<i>t</i> -Amylamine	0
<i>n</i> -Hexylamine	65
<i>n</i> -Heptylamine	60
<i>n</i> -Octylamine	45
Benzylamine	45
Phenethylamine	54
Tyramine	5
Tryptamine	20
Histamine	10

that for β-alanine. The enzyme catalyzed transamination between primary alkylmonoamines including straight- or branched-chain structure and pyruvate. The enzyme activity was affected by carbon units in length of straight-chain alkylmonoamines. A maximum activity was obtained for 5-carbon alkylmonoamine, *n*-amylamine. Branched-chain amines

exhibited a similar activity to the corresponding straight-chain amines. Secondary amine, *sec*-butylamine was active to some extent, though tertiary amines were inert, suggesting that the presence of hydrogen atom at the carbon carrying amino group is essential for the transamination. The enzyme exhibited activity against aromatic amines *i.e.* benzylamine, phenethylamine, tryptamine, tyramine and histamine to a lesser extent. The presence of hydroxyl group was inhibitory to the reaction; ethylamine and phenethylamine showed to be more active than the corresponding hydroxyl analogue *i.e.* ethanolamine and tyramine, respectively.

Specificity of amino acceptors was investigated in the reaction system containing *n*-butylamine as an amino donor. Pyruvate was an exclusive amino acceptor; α -ketoglutarate, α -ketobutyrate, oxaloacetate, glyoxylate and β -phenylpyruvate were inert. When the enzyme activity was examined in the reaction system using *n*-butylaldehyde as an amino acceptor, L-alanine was a good amino donor; D-alanine was inert, indicating that L-alanine is a product of the reaction between amine and pyruvate as shown in the transamination of ω -amino acid.

Kinetics

Effect of a change in the concentration of *n*-butylamine or pyruvate on the reaction velocity were examined by the method of Velick-Vavra¹⁴⁾ (Fig. 3). The kinetic pattern showed a ping pong bi bi mechanism for the reaction system. The same mechanism was observed for the transamination between β -alanine and pyruvate. Michaelis constant for *n*-butylamine and pyruvate were 66.6 mM and 5.5 mM, respectively. The constant for pyruvate was comparable to that obtained in the β -alanine-pyruvate system; confirming that the same enzyme catalyzes both amine and ω -amino acid transaminations.

DISCUSSION

Most of a biogenic amine are formed by a decarboxylation of L- α -amino acid catalyzed

FIG. 3. Effect of Concentrations of *n*-Butylamine and Pyruvate on the Enzyme Activity.

The reaction mixture contained 0.1 μ mol of pyridoxal-P, 100 μ mol of potassium phosphate buffer (pH 10.0), the enzyme and various amounts of the substrates. The enzyme activity was assayed by assay method B. (A): Double-reciprocal plots of initial velocity against pyruvate concentrations at a series of fixed concentrations of *n*-butylamine. $\bullet$ — $\bullet$, 15 mM; $\circ$ — $\circ$, 20 mM; $\blacktriangle$ — $\blacktriangle$, 30 mM; $\triangle$ — $\triangle$, 40 mM; $\blacksquare$ — $\blacksquare$, 50 mM. (B): Secondary plot from intercepts $1/V$ of (A). (C): Double-reciprocal plot of V against *n*-butylamine concentrations at a series of fixed concentrations of pyruvate. $\bullet$ — $\bullet$, 2.0 mM; $\circ$ — $\circ$, 3.0 mM; $\blacktriangle$ — $\blacktriangle$, 5.0 mM; $\triangle$ — $\triangle$, 8.0 mM; $\blacksquare$ — $\blacksquare$, 10.0 mM. (D): Secondary plot from the intercepts $1/V$ of (C).

by an amino acid decarboxylase, while the degradation is carried out by an amine dehydrogenase or an amine oxidase.¹⁵⁻¹⁷⁾ Therefore, studies on the enzymologic aspects of amine metabolism have been focussed on these enzymes. An aminotransferase catalyzing transamination between mono- or polyamines and α -ketoglutarate or pyruvate has been demonstrated in plant and bacteria.¹⁸⁾ Diamine- α -ketoglutarate transaminase of a mutant of *Escherichia coli* was purified and partially characterized.^{19,20)} Amine transamination can serve in the synthesis or degradation of amine, suggesting a physiological significance of the transamination for amine metabolism. There has been increasing interests in the transamination of amines. Recently the amine transamination catalyzed by ω -amino acid aminotransferases has been demonstrated. 4-Amino-

butyrate aminotransferase of *Pseudomonas aeruginosa* shows a high activity for polyamines but not for β -alanine.²¹⁾ Transamination of *n*-propylamine was demonstrated by β -alanine transaminase of *Bacillus cereus*.⁸⁾ The enzyme, however, can not react with *n*-butylamine. Taurine- α -ketoglutarate aminotransferase of *Achromobacter superficialis*, showing a high activity for various ω -amino acids such as β -alanine and γ -aminobutyrate, can not catalyze the amine transamination.⁷⁾ Diamine- α -ketoglutarate aminotransferase can not react with ω -amino acids, alkyl and aromatic monoamines.^{19,20)} ω -Amino acid: pyruvate aminotransferase of *Pseudomonas* sp. F-126 catalyzed a transamination of various amines with pyruvate besides that of ω -amino acids. Most of alkyl and aromatic monoamines, more or less, can serve as an amino donor. The enzyme is unique in this regard. Michaelis constant (66.6 mM) for *n*-butylamine was rather high value, compared with that obtained for the other enzymes participating in amine metabolism *e.g.* amine oxidase and amine dehydrogenase. The *K_m* values for the latter two enzyme are low about two orders.^{16,22,23)}

One of the interesting problems concerned with the enzyme reaction is whether a single site or separate sites of the enzyme operate in binding with amines and pyruvate. In contrast to a high substrate specificity for α -keto acid, specific for pyruvate, the enzyme catalyzed a transamination of various amines different in carbon skeleton. This result suggests that amine and pyruvate bind to separate sites of the enzyme; amine site and pyruvate site, respectively. On the basis of similarity in substrate specificity for amine and for ω -amino acid, it seems likely that both compounds interact with the same site of the enzyme.

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