Candida δ-Aminovalerate:α-Ketoglutarate Aminotransferase: Purification and Enzymologic Properties

P. Arsène Der Garabedian
Laboratoire d'enzymologie, Université Pierre et Marie Curie, UER 58, 75006 Paris, France
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ABSTRACT: A new enzyme that catalyzes the transamination of δ-aminovalerate with α-ketoglutarate was purified to homogeneity from adapted cells of *Candida guilliermondii* var. *membranaefaciens*. The relative molecular mass determined by gel filtration was estimated to be close to 118 000. The transaminase behaved as a dimer with two similar subunits in sodium dodecyl sulfate–polyacrylamide gel electrophoresis. The enzyme has a maximum activity in the pH range of 7.8–8.5 and at 40 °C. α-Ketoglutarate and to a lesser extent pyridoxal 5′-phosphate were effective protecting agents toward temperature raising. The enzyme exhibits absorption maximum at 330 and 410 nm. The enzyme catalyzes the transamination between ω-amino acids and α-ketoglutarate. δ-Aminovaleric acid is the best amino donor. The K_m values for δ-aminovalerate, α-ketoglutarate, and pyridoxal 5′-phosphate determined from the Lineweaver–Burk plot were 4.9 mM, 3.6 mM, and 22.7 μM, respectively. The inhibitory effect of various amino acid analogues on the transamination reaction between δ-aminovalerate and α-ketoglutarate was studied, and K_i values were determined.

Since the first discovery of transaminases by Braünstein and Kritzmann (1937), various other transaminases have been reported in the literature. In most cases, the amino donor substrates are aliphatic amino acids of which amino groups are in the α- or ω-position.

A few data relative to ω-aminotransamination are available in mammalian tissues (Roberts & Bregoff, 1953) and microorganisms (Roberts et al., 1953). Especially γ-aminobutyrate transaminase (Churchich, 1982; Yonaha & Toyama, 1980a,b; White & Sato, 1978; Schousboe et al., 1973; Bloch-Tardy et al., 1974; John & Fowler, 1976; Kirby et al., 1985) and β-alanine transamination (Yonaha & Toyama, 1980b; Yonaha et al., 1983, 1985a; Nishizuka et al., 1959; Hayaishi et al., 1961) have been investigated. δ-Aminovalerate transamination has been observed in beef brain (Baxter & Roberts, 1958), pig liver (Buzenet et al., 1978), mouse brain (Schousboe et al., 1973), *Pseudomonas* SP 126 (Yonaha & Toyama, 1980a), and acetone powder of brain, liver, *Escherichia coli* and *Aspergillus fumigatus* (Roberts, 1954), but only one δ-aminovalerate aminotransferase (2.6.1.48) within our knowledge has been partially purified from lysine-adapted cells of *Pseudomonas* (Ichihara et al., 1960).

δ-Aminovaleric acid is an intermediate metabolite of lysine dissimilation in the yeast *Hansenula saturnus* (Rothstein, 1965). In addition, this amino acid is involved in ornithine metabolism via proline in animals, plants, and bacteria (Adams & Franck, 1980).

Previous observations on the growth of some species of *Candida* in a medium containing ω-amino acids as sole source of nitrogen (Der Garabedian & Indzikian, 1966), served as starting point for further investigations.

In the present study, we described the purification and the enzymologic properties of δ-aminovalerate:α-ketoglutarate transaminase from *Candida guilliermondii* var. *membranaefaciens*. On the basis of the substrate specificity, the enzyme was found to be a novel ω-amino acid transaminase.

Experimental Procedures

Materials. The following reagent-grade chemicals were purchased from Sigma Chemical Co.: L-amino acids, pyridoxal 5′-phosphate, α-ketoglutarate, 2,6-dichlorophenolindophenol, phenazine methosulfate, acrylamide, *N*,*N*′-methylenebis(acrylamide), and sodium dodecyl sulfate. NAD,[1] glutamate dehydrogenase, and standard proteins for the determination of molecular weights were purchased from Boehringer (Mannheim). Ultrogel AcA34 was obtained from LKB, CM-Sephadex and DEAE-Sephadex were from Pharmacia, and Coomassie Brilliant Blue R 250 was from Kodak.

Cell Cultures. Investigations were carried out on yeast *Candida guilliermondii* var. *membranaefaciens* (Y43). Serial transfers on agar plates and an enrichment medium containing δ-aminovalerate as sole source of nitrogen resulted in the isolation of a pure culture; this was then transferred in the following liquid medium (in 1 L of bidistilled water at pH 5.5 and 35 °C): glucose, 20 g; δ-aminovalerate, 4.86 g; KH_2PO_4, 1.23 g; $MgSO_4 \cdot 7H_2O$, 0.685 g; $CaCl_2 \cdot 2H_2O$, 0.125 g; NaCl, 0.125 g; biotin, 4 μg.

The oxygenation of the liquid medium (5 l) was performed by circular stirring and bubbling in a fermenter. The yeasts were harvested at the end of the exponential phase (18 h) by centrifugation at 750*g* for 5 min at 4 °C followed by two washing with bidistilled water. The cells obtained (16 g) were immediately pulverized or lyophilized for further investigations.

The enzyme was induced by the presence of δ-aminovalerate and L-lysine in the medium; glycine, 4-aminobutyrate, and ε-aminocaproate were inactive for the enzyme induction.

Enzymatic Extract Preparation. The enzyme preparation procedure was carried out generally at about 4 °C. Fresh yeasts (7.5 g) or lyophilized yeasts (1.5 g) were treated in a MSK cell grinder (Braun) while being cooled with CO_2 gas. The grinding was performed for 10 min repeated 3 times with glass beads (33 g of beads, o.d. 0.45 mm, + 10 g of beads, o.d. 0.17 mm) in a volume (50 mL) of 0.066 M potassium phosphate buffer, pH 7.8, adequate to fill up the grinding flask.

[1] Abbreviations: NAD, nicotinamide adenine dinucleotide; DCPIP, 2,6-dichlorophenolindophenol; PMS, phenazine methosulfate; pyridoxal-5′-P, pyridoxal 5′-phosphate; α-KG, α-ketoglutarate; GIDH, glutamate dehydrogenase; SDS, sodium dodecyl sulfate; δ-Ptn, δ-aminovalerate; Gly, glycine; α-Ala, α-alanine; β-Ala, β-alanine; A_2pr, α,β-diaminopropionate (α,β-alanine); A_2bu, α,γ-diaminobutyrate; L-Orn, L-ornithine; L-Lys, L-lysine.

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The supernatant obtained after centrifugation at 900*g* for 20 min is considered as "crude extract"; the supernatant obtained after centrifugation at 33000*g* for 30 min is considered as "initial extract".

Protein Determination. Protein was assayed by the method of Lowry et al. (1951) with bovine serum albumin as the standard.

Transaminase Assays. The glutamate formed during the transamination between α-ketoglutarate and various amino acids was measured by a coupled reaction: glutamate dehydrogenase, NAD, glutamate, dichlorophenolindophenol (DCPIP), and phenazine methosulfate (PMS). Usually, the identification included the following two successive steps (Der Garabedian, 1985). The first step was glutamate synthesis in a reaction medium (1 mL) containing the following: L-amino acid, 20 μmol; pyridoxal-5′-P, 0.1 μmol; α-KG, 20 μmol; enzyme; potassium phosphate buffer, 50 μmol (pH 7.8). Incubation was performed during 7.5 min at 35 °C. The second step was measurement of the glutamate formed "de novo". A 0.1-mL aliquot of the previous medium was added to 0.9 mL of the following mixture: NAD, 0.15 μmol; DCPIP, 0.06 μmol; PMS, 0.08 μmol; GIDH, 100 μg; potassium phosphate buffer, 50 μmol (pH 7.8).

The decrease of absorbance at 600 nm was followed for 5–10 min (ΔA = 0.016 per 1 nmol of reduced DCPIP). In order to identify enzyme activity in the column eluates during the purification procedure, a less sensitive method was used. The glutamate synthesized in the first part of the previous test was quantitatively estimated by paper chromatography. An aliquot is spotted on Whatman 3MM paper. The migration is performed in the solvent mixture 1-butanol/acetic acid/H_2O (4:1:1). Glutamate is measured at 510 nm by a colorimetric method with ninhydrin (Hais & Macek, 1963). One unit of the enzyme is defined as the amount of enzyme that produces 1.0 μmol of glutamate/min, and specific activity is expressed as units per milligram of protein.

Ultrafiltration. The active enzymatic fractions were dialyzed and concentrated by ultrafiltration under nitrogen pressure (72.5 psi) in an Amicon cell with a Diaflo membrane PM-10 or PM-30 at 4 °C.

Electrophoresis. Polyacrylamide gel electrophoresis was performed according to the original procedure of Davis (1964). Disc gel electrophoresis in sodium dodecyl sulfate (SDS) was performed as described by Laemmli (1970). Relative mobilities were calculated with proteins used as markers such as catalase (M_r 60000), bovine serum albumin (M_r 67000), egg ovalbumin (M_r 45000), and chymotrypsinogen A (M_r 25000).

Results

Purification. Enzyme purification procedure was carried at about 4 °C unless otherwise specified. All buffers used from step I to step VI contained 0.01 mM pyridoxal-5′-P and 0.01 mM α-ketoglutarate.

(*Step I*) *Ammonium Sulfate Precipitation.* The "initial extract" was dialyzed against 0.01 M potassium phosphate buffer (pH 7.8) and 1 mM 2-mercaptoethanol until elimination of the substances that reacted with ninhydrin and then concentrated. Ground solid ammonium sulfate was added slowly with stirring at 0 °C over a period of 45 min to this extract until 40% saturation was reached. After equilibration for 1.5 h, the precipitate was discarded by centrifugation at 33000*g* for 75 min. The concentration of ammonium sulfate in the supernatant was raised to 83.5% saturation. The precipitate was allowed to equilibrate for 15 h and was removed by centrifugation for 2 h at 215000*g* (Beckman L5-65). The pellet was suspended in 0.05 M potassium phosphate buffer (pH 7.8), dialyzed against the same buffer, and concentrated.

Table I: Purification of δ-Aminovalerate Transaminase from Yeast

purification step	total protein (mg)	total act. (units)	sp act. (units/mg)	purification (*x*-fold)
crude extract	3000	150	0.05	1
initial extract	687	103	0.15	3
(I) $(NH_4)_2SO_4$, 40.0–83.5% sat.	208	76.9	0.37	7.5
(II) Ultrogel AcA34	109	59.9	0.55	11
(III) CM-Sephadex C-50	44.5	49.8	1.12	22.5
(IV) DEAE-Sephadex A-50	15.6	36.6	2.35	47
(V) Ultrogel AcA34	6.2	32.2	5.2	104
(VI) preparative electrophoresis	0.583	12.12	20.8	416

(*Step II*) *Ultrogel AcA34 Chromatography.* The ammonium sulfate extract was loaded onto an Ultrogel AcA34 column (2.6 × 90 cm) equilibrated with 0.05 M potassium phosphate buffer (pH 7.8) and 10 mM KCl. Proteins were eluted with the same buffer, at a rate of 19.6 mL/h.

(*Step III*) *CM-Sephadex C-50 Chromatography.* The active protein fractions of step II were pooled, dialyzed against 0.01 M potassium phosphate buffer (pH 6.5), and concentrated. This fraction was applied on a CM-Sephadex C-50 (1.6 × 15 cm) column, previously equilibrated, and eluted by a 0.01–0.05 M linear potassium phosphate buffer gradient. The fractions that were active with most amino acids were not bound to the CM-Sephadex C-50, indicating that the enzyme had a net negative charge at pH 6.5.

(*Step IV*) *DEAE-Sephadex A-50 Chromatography.* The active fractions of step III were pooled, dialyzed against 0.01 M potassium phosphate buffer (pH 7.8) and 50 mM KCl, and concentrated by ultrafiltration. The collected fractions were transferred to a DEAE-Sephadex A-50 column (1.6 × 15 cm), preequilibrated with the above dialysis buffer, and eluted with a linear KCl gradient (0.05–0.5 M, pH 7.8). The δ-aminovalerate activity was eluted at about 0.35 M KCl, pH 7.8.

(*Step V*) *Ultrogel AcA34 Rechromatography.* The pooled enzymatic fractions were dialyzed against a 0.05 M potassium phosphate buffer (pH 7.8) and 10 mM KCl. After concentration, the enzyme solution was subjected to a second Ultrogel AcA34 column (1.6 × 120 cm) previously equilibrated with the dialysis buffer. Elution was carried out with the same buffer. The active protein fractions were dialyzed against the elution buffer without KCl and concentrated before the last step of purification.

(*Step VI*) *Preparative Polyacrylamide Gel Electrophoresis.* Protein analysis of the enzyme from step V on polyacrylamide gel enabled us to detect three major bands. The δ-aminovalerate activity is localized on a single main band that represents about 35% of the proteins applied onto the gel. Slices of polyacrylamide gel containing the active band were incubated with 0.05 M potassium phosphate buffer to extract the transaminase from the gel. At this point, the purification factor was about 416-fold with respect to the "crude extract". The protein was lyophilized and stored at −20 °C. A summary of the purification procedure of the enzyme is given in Table I.

Apparent Molecular Weight. The purified enzyme was shown to be homogeneous. The relative molecular mass of δ-aminovalerate transaminase was determined by exclusion–diffusion chromatography on an Ultrogel AcA34 (1.0 × 68 cm) column. Comparison of its R_f with those of several standard proteins yielded a molecular mass of 118000. On

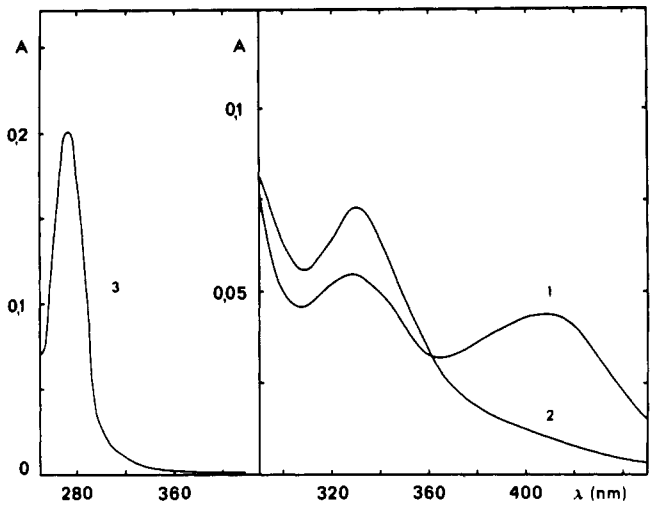

FIGURE 1: Spectrum absorption of δ-aminovalerate transaminase: (curve 1) holoenzyme; (curve 2) holoenzyme with δ-aminovaleric acid; (curve 3) apoenzyme. The enzymatic solution (0.25 mg of protein/mL) was in a 0.05 M potassium phosphate buffer, pH 7.8.

the other hand, a molecular mass of about 60 000 was found in SDS–polyacrylamide (8.75 %) gel, suggesting that the enzyme was a dimer of two identical subunits.

Absorption Spectra and Pyridoxal 5′-Phosphate Content. Spectra were recorded at 20 °C. The absorption spectrum of the transaminase from *Candida* is presented in Figure 1.

The enzyme exhibits maximum absorption at 410 nm showing a typical Schiff base formed between the enzyme protein (A = 280 nm) and pyridoxal-5′-P and a low absorbance around 330 nm (curve 1). Addition of the amino donor (δ-aminovalerate) caused a decrease in the absorbance at 410 nm with a concomitant increase in the 330-nm absorbance (curve 2). This demonstrates the conversion of the coenzyme from its pyridoxal form to its pyridoxamine form. Addition of α-ketoglutarate reversed the decrease in the absorbance at 410 nm, but did not affect absorbance at 330 nm.

The enzyme was treated with 25 mM H_2SO_4 at 120 °C for 2 h in order to release the enzyme-bound pyridoxal-5′-P: the liberated coenzyme was determined to be 2 mol of pyridoxal-5′-P/mol of enzyme by the phenylhydrazine method (Wada & Snell, 1961).

Curve 3 represents the spectrum of the apoenzyme obtained by the method described by Sanada et al. (1976). The resulting apoenzyme is inactive but regains more than 90% of its original enzymatic activity after addition of pyridoxal-5′-P.

Substrate Specificity. The specificity of the purified enzyme for various amino donors was studied by using the standard assay system, in which amino acids were substituted for δ-aminovalerate (Table II). All measurements of δ-aminotransferase activity were done with α-ketoglutarate as the amino acceptor.

The highest activity was obtained with δ-aminovaleric acid. The enzyme catalyzed transamination with 4-aminobutyrate to a lesser extent and a low activity was observed for glycine, L-leucine, and L-norleucine. All of the other amino acids tested were completely inert.

Effect of pH and Temperature. The pH dependence of transamination between δ-aminovalerate and α-ketoglutarate was examined in a 0.05 M potassium phosphate buffer from pH 6.5 to pH 9.0 at 35 °C: maximum activity is observed at pH 7.8–8.5.

The aminotransferase reaction was carried out at various temperatures in the standard reaction system at pH 7.8. The reaction velocity linearly increased with temperature from 25 to 40 °C, and the activity rapidly decreased above 40 °C (data not shown). Finally, the enzyme was completely inactive at 60 °C.

Table II: Substrate Specificity[a]

amino donor[b]	relative act.
δ-aminovalerate	100
4-aminobutyrate	40
glycine	1
L-leucine	10
L-norleucine	4.8

[a] α-Aminovalerate, α-aminobutyrate, D-ornithine, L-ornithine, L-lysine, α,γ-aminobutyrate, α-alanine, β-alanine, α,β-alanine, L-valine, ε-aminocaproate, 7-aminocaproate, L-methionine, L-tyrosine (0.12 mM), L-aspartate, L-arginine, phenylalanine, glutamine, asparagine, cysteine, and *S*-methylcysteine are inert. [b] Amino donor concentration = 10 mM unless otherwise specified.

In another set of experiments, the enzyme was incubated at 50 °C in the presence of α-ketoglutarate or pyridoxal-5′-P, or without any protecting agent for different times, and the enzymatic reaction was performed as described under Experimental Procedures, with the heated enzyme. At 50 °C, it appeared that the transaminase preserved 50% of its activity after being heated for 7 min in the presence of α-ketoglutarate, after 4 min in the presence of pyridoxal-5′-P, and after 1 min without α-KG or pyridoxal-5′-P. This result shows that α-ketoglutarate is the best protecting agent.

Michaelis Constant. Apparent K_m values for α-ketoglutarate and δ-aminovalerate were determined either by varying concentrations of δ-aminovalerate at several concentrations of α-ketoglutarate or by varying concentrations of α-ketoglutarate at fixed concentrations of δ-aminovalerate. Figure 2 indicated a Ping-Pong-Bi-Bi mechanism for the enzyme reaction. The K_m values for α-ketoglutarate and δ-aminovalerate were determined to be 3.6 mM and 4.9 mM, respectively.

The K_m value for pyridoxal-5′-P has been determined either by varying concentrations of δ-aminovalerate for several fixed concentrations of α-ketoglutarate or by varying concentrations of α-ketoglutarate for several fixed concentrations of δ-aminovalerate. The K_m value for pyridoxal-5′-P was calculated to be 2.27×10^{-5} M.

Effect of Various δ-Aminovalerate Analogues on Transaminase Activity. Figure 3 shows the inhibition of L-ornithine with respect to δ-aminovalerate (mixed) and α-ketoglutarate (competitive). Kinetic constants of inhibition are 9.5 mM and 5.6 mM, respectively.

Glycine, α-alanine, β-alanine, α,β-alanine, α,γ-aminobutyrate, and L-lysine exhibit a noncompetitive inhibition with respect to δ-aminovalerate and a competitive inhibition with respect to α-ketoglutarate. Figure 4 shows an example of these types of inhibition for α,γ-aminobutyrate. K_i values for the various amino acid inhibitors studied and for α-ketoglutarate together with the type of inhibition are presented in Table III.

Discussion

A considerable effort has been devoted to the characterization of ω-amino acid transaminases, key enzymes involved in ω-amino acid metabolism, in recent years, but only one δ-aminovalerate transaminase (2.6.1.48) has been identified from *Pseudomonas* (Ichihara et al., 1960).

The results reported in this study show that a purified homogeneous preparation of a new induced δ-aminovalerate transaminase from the yeast *C. guilliermondii* var. *membranaefaciens* accepts δ-aminovaleric acid as a main substrate with α-ketoglutaric acid as amino acceptor. γ-Aminobutyrate is to a lesser extent an effective amino donor in the reaction

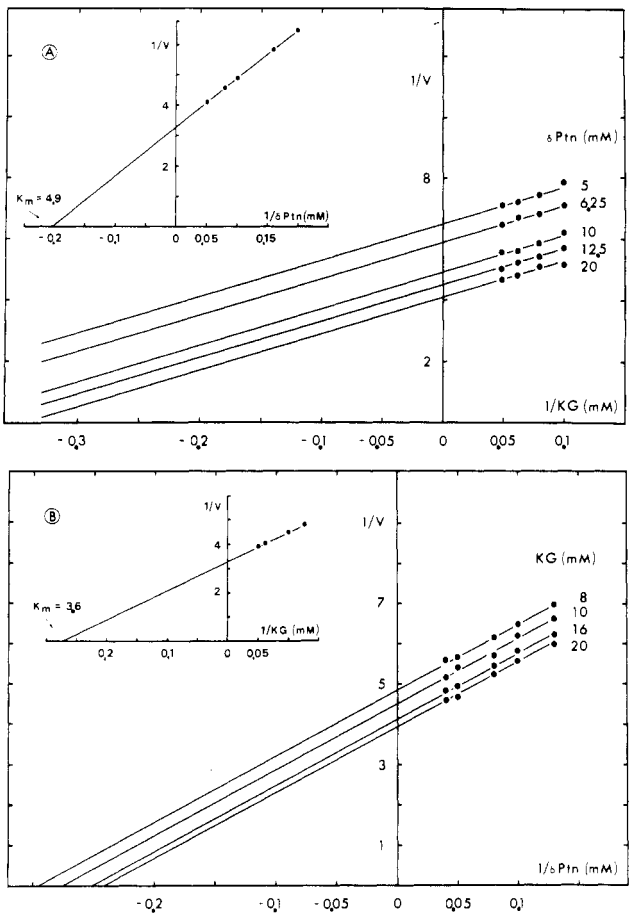

FIGURE 2: Transamination of δ-aminovalerate and α-ketoglutarate and determination of K_m values. Effect of concentrations of α-ketoglutarate and δ-aminovalerate on the enzyme activity. (A) Double-reciprocal plots of initial velocity against α-ketoglutarate (KG) concentration at a series of fixed concentrations of δ-aminovalerate (δ-Ptn). (Inset) Secondary plots from the intercepts of $1/V$ of (A). Determination of K_m of δ-Ptn (4.9 mM). (B) Double-reciprocal plots of initial velocity against δ-aminovalerate (δ-Ptn) concentrations at a series of fixed concentrations of α-ketoglutarate (KG). (Inset) Secondary plots from the intercepts $1/V$ of (B). Determination of K_m of KG (3.6 mM).

Table III: Kinetic Constants of Inhibition of δ-Aminovalerate Aminotransferase from *Candida*

amino acid analogues[a]	K_i (mM)[b]	K_i (mM)[c]
Gly	90 ± 4.5	10 ± 0.5
α-Ala	120 ± 6.3	15 ± 0.7
β-Ala	16 ± 0.8	5 ● 0.2
A_2pr	50 ± 3.0	7 ± 0.3
A_2bu	100 ± 6.0	11.5 ± 0.6
L-Orn	9.5 ± 0.5	5.6 ± 0.3
L-Lys	120 ± 6.0	12.5 ± 0.6

[a] α-Aminobutyrate, 4-aminobutyrate, α-aminovalerate, and ε-aminocaproate did not cause any inhibition on the δ-aminovalerate transamination. [b] Inhibitory constants of amino acid analogues that cause noncompetitive inhibitions (excepted L-ornithine for which a mixed inhibition is observed) with respect to δ-aminovalerate. [c] Inhibitory constants of amino acid analogues with respect to α-ketoglutarate that cause competitive inhibition.

catalyzed by the enzyme, whereas β-alanine shows weak activity.

The transaminase described in this paper is a dimer of two subunits identical in molecular weight. Its relative molecular mass was estimated to be 118 000. Its optimum pH is 7.8–8.5. This value is lower than the optimum (pH 9) found for the other induced δ-aminovalerate transaminase known, from

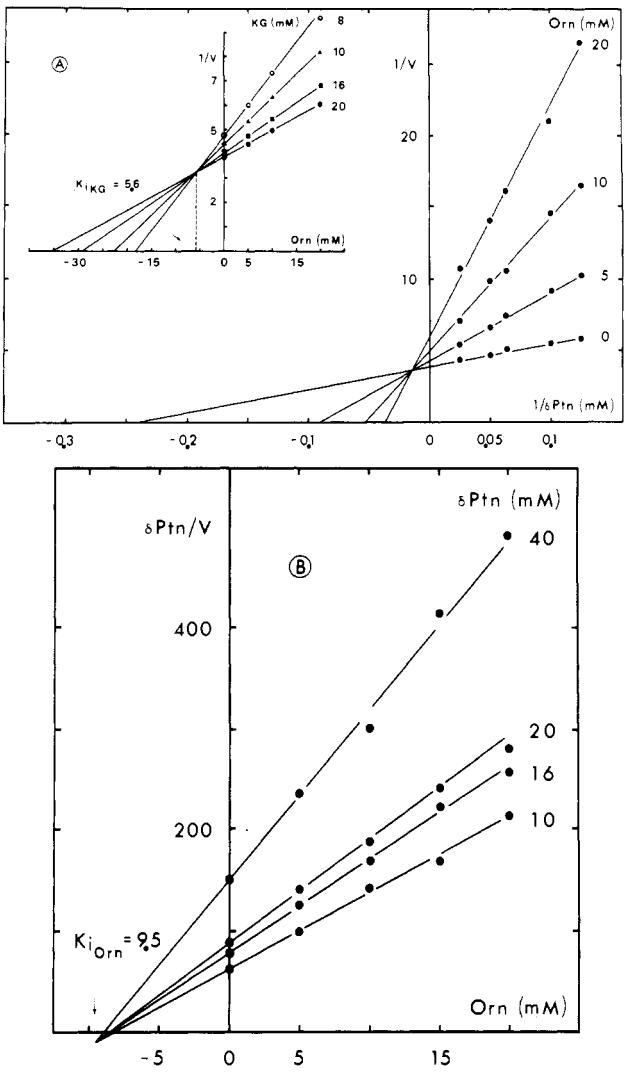

FIGURE 3: Inhibition of δ-aminovalerate transaminase by L-ornithine. (A) Lineweaver–Burk representation for the determination of L-ornithine inhibition (mixed) with respect to δ-aminovalerate. $1/V$ vs. $1/[\delta\text{-Ptn}]$ at different fixed L-ornithine concentrations and a constant α-ketoglutarate concentration (20 mM). (Inset) Dixon representation for the determination of L-ornithine competitive inhibtion and K_i value with respect to α-ketoglutarate. $1/V$ axis intercept replot from (A). Each replot represents a different α-ketoglutarate concentration. (B) Cornish–Bowden representation for the determination of L-ornithine K_i value with respect to δ-aminovalerate. $[\delta\text{-Ptn}]/V$ vs. [L-ornithine] at different fixed δ-aminovalerate concentrations.

Pseudomonas (Ichihara et al., 1960).

As previously demonstrated for other types of transaminases, α-ketoglutarate and pyridoxal-5′-P protect the enzyme against thermal denaturation, α-ketoglutarate being better than pyridoxal-5′-P (Chesne & Pelmont, 1974; Baxter & Roberts, 1958).

The absorption spectrum of the enzyme shows maxima at 280, 330, and 410 nm. The addition of δ-aminovalerate caused a decrease in the 410-nm peak and an increase in the 330-nm peak. Decrease in absorbance at 410 nm was reversed by addition of α-ketoglutarate, suggesting that the 410-nm peak is directly involved in the catalysis. On the contrary, absorbance at 330 nm is not affected by amino acceptor, indicating that the 330-nm-absorbing coenzyme does not directly participate in the reaction.

The enzyme from yeast possesses spectral characteristics that suggest that the binding of pyridoxal-5′-P to the apoenzyme is established on the same principles as in some other

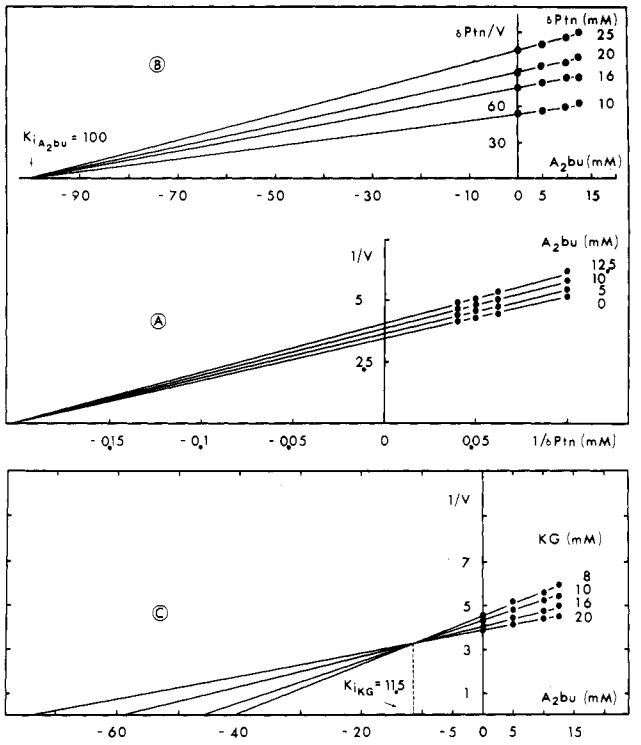

FIGURE 4: Inhibition of δ-aminovalerate transaminase by α,γ-aminobutyrate. (A) Lineweaver–Burk representation for the determination of α,γ-aminobutyrate inhibition (noncompetitive) with respect to δ-aminovalerate. $1/V$ vs. $1/[\delta\text{-Ptn}]$ at different fixed α,γ-aminobutyrate concentrations and a constant α-ketoglutarate concentration (20 mM). (B) Cornish–Bowden representation for the determination of α,γ-aminobutyrate K_i value with respect to δ-aminovalerate. $[\delta\text{-Ptn}]/V$ vs. α,γ-aminobutyrate at different fixed δ-aminovalerate concentrations. (C) Dixon representation for the determination of α,γ-aminobutyrate competitive inhibition and K_i value with respect to α-ketoglutarate. [$1/V$ axis intercept replot from (A).] Each replot represents a different α-ketoglutarate concentration.

transaminases (Martinez-Carrion & Jenkins, 1965; Taylor & Jenkins, 1966; Fasella, 1967; Martinez-Carrion et al., 1967; Matsuzawa et al., 1968; Soda & Misono, 1968; Peraino et al., 1969; Yonaha et al., 1975; John & Fowler, 1976; Beeler & Churchich, 1978; John et al., 1979; Moses & Churchich, 1980; Yonaha et al., 1985b; Kirby et al., 1985).

The *Candida* δ-aminovalerate transaminase contains 2 mol of pyridoxal-5′-P and consists of two subunits. It is therefore likely that 1 mol of pyridoxal-5′-P binds to each subunit as also shown for *Streptomyces griseus* 4-aminobutyrate transaminase (Yonaha et al., 1985b).

The K_m values were determined to be 4.9 mM and 3.6 mM for δ-aminovalerate and α-ketoglutarate, respectively. The K_m value for pyridoxal-5′-P was 2.27×10^{-5} M, which is nearly the same as those found for other transaminases (Hayaishi & Nishizuka, 1962; Tamino, 1964; Dempsey & Snell, 1963; Ichihara & Koyama, 1966).

Various amino acids were tested for their inhibitory effects on the transamination between δ-aminovalerate and α-ketoglutarate. L-Ornithine, which has the same carbon skeleton as δ-aminovalerate, has the best inhibitory effect on δ-aminovalerate transaminase (mixed inhibition, K_i = 9.5 mM). In the serie of ω-amino acids studied, β-alanine (non competitive inhibition, K_i = 16 mM) is nearly as effective inhibitor as L-ornithine, whereas 4-aminobutyrate and ε-aminocaproate are not inhibitors. Aliphatic diamino acids such as α,β-alanine, α,γ-aminobutyrate, and L-lysine are less effective inhibitors (noncompetitive inhibition), and their K_i values are increasing with the length of their carbon chain. α-Monoamino acids are also weak inhibitors (glycine and α-alanine) or not inhibitors (α-aminobutyrate). The inhibition observed for α-ketoglutarate is competitive in all cases studied.

The physiological role of δ-aminovalerate transaminase, however, is not only defined by its substrate specificity but also by the regulation of its synthesis. The enzyme is strongly induced in cells grown on δ-aminovalerate or on lysine as the only nitrogen source (Der Garabedian, 1985).

The induced synthesis of δ-aminovalerate transaminase, by δ-aminovalerate or lysine in *Candida*, its purification, and its properties may give additional data relative to the connection that exists between ω-transaminases and the major metabolic pathways of amino acids, especially enabling us to elucidate one of the poorly studied metabolic pathways involving lysine and δ-aminovalerate (Rothstein, 1965) and the true role of δ-aminovaleric acid as an intermediary metabolite.

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Registry No. δ-Ptn, 660-88-8; α-KG, 328-50-7; A_2pr, 515-94-6; A_2bu, 305-62-4; α-Ala, 56-41-7; Gly, 56-40-6; β-Ala, 107-95-9; L-Orn, 70-26-8; L-Lys, 56-87-1; pyridoxal-5′-P, 54-47-7; δ-Ptn aminotransferase, 37277-97-7.

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Unique Rearrangement of Ergocalciferol Side Chain in Vitro: Production of a Biologically Highly Active Homologue of 1,25-Dihydroxyvitamin D_3[†]

Yoko Tanaka,[‡] Rafal R. Sicinski,[§] and Hector F. DeLuca*
Department of Biochemistry, College of Agricultural and Life Sciences, University of Wisconsin—Madison, Madison, Wisconsin 53706
Hiroshi Sai and Nobuo Ikekawa
Department of Chemistry, Tokyo Institute of Technology, Tokyo 152, Japan
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ABSTRACT: In vitro incubation of 24-epi-25-hydroxyvitamin D_2 with chicken kidney homogenate produced several compounds, one of which had an affinity equal to that of 1,25-dihydroxyvitamin D_2 for the chick intestinal receptor. The affinity of 24-epi-1,25-dihydroxyvitamin D_2 for the same receptor was found to be half that of 1,25-dihydroxyvitamin D_2. The unknown compound was produced only when homogenate was prepared from pooled kidneys taken from both vitamin D deficient and replete chickens. The compound has been tentatively identified as 1,25-dihydroxy-22-dehydro-26-homovitamin D_3 by ultraviolet absorption spectrophotometry and mass spectrometry. Chemical synthesis of 1,25-dihydroxy-22-dehydro-26-homovitamin D_3 provided additional evidence for the structure. Administration of this 26-homologue of 1,25-dihydroxyvitamin D_3 at the dose level of 650 pmol/rat stimulated bone calcium mobilization in the hypocalcemic rat equal to that of 1,25-dihydroxyvitamin D_3. Thus, this paper demonstrates unique methyl migration on the side chain of 24-epi-1,25-dihydroxyvitamin D_3 to form a more biologically potent analogue.

Vitamin D_2 undergoes the same functional metabolism as vitamin D_3, namely, 25-hydroxylation in liver and 1-hydroxylation in kidney, to exert its activity on the target tissues (Jones et al., 1976a,b). Biological potencies of vitamin D_2 and/or its metabolites are equal to that of vitamin D_3 in the rat (Jones et al., 1975; Suda et al., 1970) and in humans (Jones et al., 1954), suggesting that the ergocalciferol side chain, which is unsaturated at C_{22}–C_{23} and has a methyl group at C_{24}, is of equal acceptability for the expression of vitamin D activity in these species. In the bird, however, vitamin D_2 and its metabolites are about one-tenth as active as those derived from vitamin D_3 (Chen & Bosmann, 1964; Drescher et al., 1969; Hibberd & Norman, 1969; Jones et al., 1976a,b). Accumulating data suggest that the differential biological activity of vitamin D_2 in the bird is due to the presence of a 24-methyl rather than an unsaturated side chain (DeLuca et al., 1968). In order to study the functional importance of 24-methyl of vitamin D_2, 25-hydroxyvitamin D_2 (25-OH-D_2) and its C-24 epimers were synthesized in the authors' laboratory (Morzycki et al., 1984) and their biological activities and metabolism studied. It was found that 24-epi-25-OH-D_2

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[‡] Present address: VA Medical Center, Albany, NY 12208.
[§] Present address: Department of Chemistry, University of Warsaw, 02-093 Warsaw, Poland.

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