Stereochemistry of Proton Abstraction Catalyzed by Lysine and Ornithine ω-Aminotransferases†

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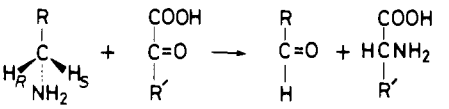
ABSTRACT: (6*R*)-L-[6-^{3}H]Lysine and (6*S*)-L-[6-^{3}H]lysine were prepared to investigate the stereochemical aspect of the reaction catalyzed by bacterial L-lysine ε-aminotransferase. When (6*R*)-L-[6-^{3}H]lysine was used as a substrate, the tritium label was retained in the product, Δ^1-piperideine-6-carboxylate. In contrast, the radioactivity from (6*S*)-L-[6-^{3}H]lysine was found in the solvent. Thus, the *pro-S* hydrogen at the prochiral C-6 carbon of L-lysine is specifically abstracted by L-lysine ε-aminotransferase. The proton exchange was observed by proton nuclear magnetic resonance analysis in the reaction of bacterial L-ornithine δ-aminotransferase with L-ornithine in ^2H_2O. The isolated L-[5-^{2}H]ornithine was converted to dextrorotatory 4-phthalimido[4-^{2}H]butyrate. This indicates that L-ornithine δ-aminotransferase catalyzes the stereospecific exchange of the *pro-S* hydrogen at the prochiral C-5 carbon of L-ornithine with the solvent.

Aminotransferase reactions proceed through the abstraction of a proton from the Schiff base complex formed between the enzyme-bound pyridoxal-P[1] and a substrate to produce an anionic quinoid intermediate (Braunstein, 1973). Substrates of α-aminotransferases except glycine have only a single α proton to be removed during transamination, whereas those of ω-aminotransferases have two chemically identical hydrogen atoms on the prochiral distal carbon (Scheme I). Thus, in the ω-aminotransferase reactions, there are two stereochemical possibilities for the proton abstraction: the stereospecific abstraction of the *pro-R* and *pro-S* hydrogens and alternatively their indiscriminate removal. On the basis of the substrate stereospecificity of α-aminotransferases, one of these ω hydrogens is expected to be removed stereospecifically by ω-aminotransferases. It has been reported recently that the bacterial ω-amino acid:pyruvate aminotransferase removes only the 4-*pro-R* hydrogen of γ-aminobutyrate (Burnett et al., 1979), whereas the bacterial and mammalian γ-aminobutyrate aminotransferases abstract the 4-*pro-S* hydrogen specifically (Burnett et al., 1979; Bouclier et al., 1979).

We have purified and crystallized four bacterial ω-aminotransferases: L-lysine ε-aminotransferase (EC 2.6.1.36; Soda & Misono, 1968), taurine:α-ketoglutarate aminotransferase (EC 2.6.1.55; Toyama et al., 1972), ω-amino acid:pyruvate aminotransferase (EC class 2.6.1; Yonaha et al., 1976), and L-ornithine δ-aminotransferase (EC 2.6.1.13; Yasuda et al., 1979), to characterize them. L-Lysine ε-aminotransferase and L-ornithine δ-aminotransferase are unique among aminotransferases: they are specific for L-amino acids but act on the distal amino group to the prochiral carbon atom. The products, L-α-aminoadipic δ-semialdehyde and L-glutamic γ-semialdehyde, in both enzyme reactions are spontaneously converted into the intramolecularly dehydrated form, Δ^1-piperideine-6-carboxylate and Δ^1-pyrroline-5-carboxylate, respectively; the reactions are virtually irreversible. In order to obtain information on the orientation of substrates in the active site of these enzymes, we have investigated stereochemistry of the proton abstraction by L-lysine ε-aminotransferase and L-ornithine δ-aminotransferase. We here report that both the ω-aminotransferases abstract stereospecifically the *pro-S* hydrogen atom from the ω-amino group bearing carbon atoms of their amino donors.

Scheme I: Reaction of ω-Aminotransferases



where R = $-(CH_2)_2CH(NH_2)COOH$; ornithine
= $-(CH_2)_3CH(NH_2)COOH$; lysine
= $-(CH_2)_2COOH$; γ-aminobutyrate

Experimental Procedures

Materials. NADP$^+$ was purchased from Kyowa Hakko Kogyo, Tokyo; DL-[2-^{3}H]glutamate (1.4 mCi/μmol) and L-[U-^{14}C]lysine (0.34 mCi/μmol) were from Radiochemical Centre, Amersham; ^3H_2O (1 mCi/g) was from New England Nuclear; ^2H_2O (99.75 atom % ^{2}H) was from Merck; glutamate dehydrogenase of bovine liver (type II) was from Sigma; Dowex 1-X8 and 50-X8 were from Dow Chemicals. *meso*-DAP was synthesized according to the method of Roy & Karel (1973) and separated from the D and L isomers as described by Wade et al. (1957). *o*-Aminobenzaldehyde was obtained by reduction of *o*-nitrobenzaldehyde (Smith & Opie, 1955). L-α-Amino-ε-ketopimelate was prepared from *meso*-DAP with *meso*-DAP D-dehydrogenase as described previously (Misono et al., 1979). [4-^{3}H]NADPH (1.23×10^7 dpm/μmol) was synthesized from DL-[2-^{3}H]glutamate and NADP$^+$ with glutamate dehydrogenase (Misono & Soda, 1980a). L-Lysine ε-aminotransferase of *Flavobacterium lutescence* (*Achromobacter liquidum*) IFO 3084 (Soda & Misono, 1968), L-ornithine δ-aminotransferase of *Bacillus sphaericus* IFO 3525 (Yasuda et al., 1979), *meso*-DAP decarboxylase of *B. sphaericus* IFO 3525 (Asada et al., 1981a), *meso*-DAP D-

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[1] Abbreviations: pyridoxal-P, pyridoxal 5′-phosphate; NADP$^+$, oxidized nicotinamide adenine dinucleotide phosphate; DAP, α,ε-diaminopimelate; NADPH, reduced nicotinamide adenine dinucleotide phosphate; Tris, tris(hydroxymethyl)aminomethane; Cl_3CCOOH, trichloroacetic acid; p^{2}H, the glass electrode reading of pH in deuterium oxide without correction for isotope effects; NMR, nuclear magnetic resonance; ppm, parts per million.

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dehydrogenase of *B. sphaericus* IFO 3525 (Misono & Soda, 1980b), and L-lysine α-oxidase of *Trichoderma viride* Y244-2 (Kusakabe et al., 1980) were purified to homogeneity as described previously. The other chemicals were analytical grade reagents.

Preparation of (6*R*)-L-[6-^3H]*Lysine.* This was synthesized from *meso*-DAP with *meso*-DAP decarboxylase in ^3H_2O as follows. *meso*-DAP (30 mg) was dissolved in 1 mL of ^3H_2O (1 mCi/g) containing 0.2 μmol of pyridoxal-P and 320 μmol of potassium phosphate buffer (pH 6.8). After addition of *meso*-DAP decarboxylase (3.26 units), the reaction mixture was incubated at 37 °C for 2 h with stirring. The reaction was stopped by addition of 0.1 mL of 4 N HCl, and the supernatant solution obtained by centrifugation was chromatographed on a Dowex 50-X8 column (200–400 mesh, H^+ form, 1 × 6 cm). After the column was washed with 0.3 N HCl, (6*R*)-L-[6-^3H]lysine was eluted with 1 N NH_4OH and assayed by the method of Soda et al. (1978) with L-lysine ε-aminotransferase. The fractions which contained the product were pooled and evaporated to dryness. The purity of the product was confirmed with a Hitachi 835 automatic amino acid analyzer. The specific radioactivity of the product was determined to be 1140 dpm/μmol with a Packard Tri-Carb 3320 liquid scintillation spectrometer in a toluene system (Bray, 1960).

Preparation of (2*R*,6*S*)-*meso*-[2-^3H]*DAP.* L-α-Amino-ε-ketopimelate (27.3 μmol) was reductively aminated by *meso*-DAP D-dehydrogenase in 40 mL of 0.15 M Tris-HCl buffer (pH 7.5) containing 2.7 μmol of [4-^3H]NADPH (3.3 × 10^7 dpm), 65 μmol of NADPH, 6 mmol of NH_4Cl, and 500 units of *meso*-DAP D-dehydrogenase. After incubation at 37 °C for 1 h, the reaction was terminated by addition of 1.6 mL of 6 N HCl. The supernatant solution obtained by centrifugation was applied to a Dowex 1-X8 column (200–400 mesh, $HCOO^-$ form, 1.8 × 10 cm), and (2*R*,6*S*)-*meso*-[2-^3H]DAP was eluted with water, which after concentration was further purified by preparative-scale thin-layer chromatography on a Merck $60F_{254}$ silica gel plate (thickness 2.0 mm) with a solvent system of propanol–water (7:3 v/v). (2*R*,6*S*)-*meso*-[2-^3H]DAP (118 760 dpm/μmol) eluted from the plate with 2 mL of 0.02 N HCl gave a single peak upon amino acid analysis.

Preparation of (6*S*)-L-[6-^3H]*Lysine.* (2*R*,6*S*)-*meso*-[2-^3H]DAP (3.35 μmol, 4 × 10^5 dpm) in 1.5 mL of 0.3 M potassium phosphate buffer (pH 6.5) containing 1 μmol of pyridoxal-P was decarboxylated with 6.52 units of *meso*-DAP decarboxylase at 37 °C for 3 h. After addition of 40 mg of the carrier L-lysine hydrochloride, (6*S*)-L-[6-^3H]lysine produced (5720 dpm/μmol) was purified as described above for the preparation of (6*R*)-L-[6-^3H]lysine.

Transamination of (6*R*)-L-[6-^3H]*Lysine or* (6*S*)-L-[6-^3H]*Lysine with α-Ketoglutarate by* L-*Lysine ε-Aminotransferase. Procedure A.* The reaction mixture contained 300 units of L-lysine ε-aminotransferase, 50 μmol of sodium α-ketoglutarate, 0.1 μmol of pyridoxal-P, 50 μmol of potassium phosphate buffer (pH 8.0), and 67.5 μmol (76 950 dpm) of (6*R*)-L-[6-^3H]lysine or 5.88 μmol (33 600 dpm) of (6*S*)-L-[6-^3H]lysine in a final volume of 1.0 mL. It was incubated at 37 °C for 1 h, followed by addition of 0.1 mL of 50% Cl_3CCOOH. After removal of Cl_3CCOOH and the remaining α-ketoglutarate by extraction with ether, the reaction mixture was concentrated to dryness. The radioactivity of tritium released into the solvent was measured in an aliquot of the evaporated water. The residue was washed with water by repeated dissolutions and evaporations and finally dissolved into 50 μL of 1 N formate. The reaction products were separated by descending paper electrophoresis at 1500 V for 1 h with 1 N formate as a solvent (Soda et al., 1968). Δ^1-Piperideine-6-carboxylate and L-glutamate were determined with the *o*-aminobenzaldehyde reagent and the ninhydrin reagent, respectively (Soda et al., 1968), and their radioactivity was measured by counting a piece of the paper.

Procedure B. The above reaction system was employed except that both L-[U-^{14}C]lysine (3.2 μmol, 240 000 dpm) and (6*R*)-L-[6-^3H]lysine (61 μmol, 69 540 dpm) were incubated with the enzyme, and the radioactivity ratio ($^{14}C/^3H$) in Δ^1-piperideine-6-carboxylate was determined. Other procedures were as described for procedure A.

L-*Ornithine δ-Aminotransferase Reaction in* ^2H_2O. L-Ornithine δ-aminotransferase (3 mg, 150 units) was dissolved in 3 mL of ^2H_2O, and H_2O was removed by repeated concentrations with an Amicon 202 ultrafiltration unit and dilutions with 10 mM potassium phosphate buffer in ^2H_2O (p^{2}H 8.0) containing 0.1 mM pyridoxal-P and 0.01% 2-mercaptoethanol. The solution containing 500 mg of L-ornithine hydrochloride, 76.1 mg of potassium α-ketoglutarate, 2 μmol of pyridoxal-P, and 1 mmol of potassium phosphate buffer (p^{2}H 8.0) also was freed from exchangeable protons by repeated evaporations and dissolutions in ^2H_2O and adjusted to a final volume of 20 mL with ^2H_2O. The reaction was started by addition of the enzyme solution (0.3 mL) and carried out at 37 °C for 48 h. After acidification of the reaction mixture to about pH 2 with 2 N HCl and centrifugation, the supernatant solution was applied to a Dowex 50-X8 column (200–400 mesh, H^+ form, 2 × 10 cm). The column was washed with 250 mL of 0.2 N HCl, and L-[5-^2H]ornithine was eluted in 5-mL fractions with a gradient system of 200 mL of 0.3 N HCl and 200 mL of 3 N HCl. The fractions positive to a ninhydrin test were pooled and evaporated to dryness. The purity of the product was confirmed by amino acid analysis. The yield was approximately 450 mg (90%). The ^1H NMR spectrum of the product in ^2H_2O taken with a JEOL FX-100 recording spectrometer operated at 100 MHz and with sodium 3-(trimethylsilyl)propionate-d_4 as an internal standard showed that a half of two hydrogen atoms at the C-5 position (δ 3.05) of L-ornithine was exchanged for deuterium (49.7 atom % excess ^2H).

Conversion of L-[5-^2H]*Ornithine into 4-Phthalimido*[4-^2H]*butyrate.* To 20 mL of 50 mM potassium phosphate buffer (pH 8.3) containing 400 mg of L-[5-^2H]ornithine and 0.3 mL of 30% H_2O_2, 14 mg of L-lysine α-oxidase (950 units) was added, and the mixture was incubated at 37 °C for 12 h with vigorous stirring. The reaction was stopped by addition of 2 mL of 6 N HCl, and the precipitated protein was removed by centrifugation. 4-Amino[4-^2H]butyrate produced was purified by Dowex 50-X8 column chromatography as described above (yield 160 mg) and converted to 4-phthalimido[4-^2H]butyrate (Hoffmann & Schiff-Shenhav, 1962). The product was crystallized from aqueous ethanol (yield about 80 mg), and its ^1H NMR spectrum in $CDCl_3$ showed the presence of nearly one deuterium atom (48.7 atom % excess ^2H) at the C-4 position (δ 3.75) of 4-phthalimidobutyrate. The phthalimide derivative thus obtained was submitted for the measurement of optical rotation with a Perkin-Elmer 241 polarimeter with a 10-cm light path.

Results

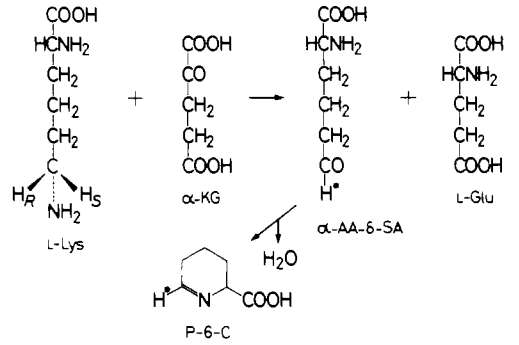
Stereospecificity for Proton Abstraction by L-*Lysine ε-Aminotransferase.* The stereospecificity for proton abstraction from the C-6 carbon of L-lysine in the L-lysine ε-aminotransferase reaction (Scheme II) was examined with two

Table I: Stereospecificity for Proton Abstraction by L-Lysine ε-Aminotransferase[a]

substrate	sp act. (dpm/μmol)	product	total act. (dpm)	sp act. (dpm/μmol)
(6*R*)-L-[6-^{3}H]lysine	1140	Δ^1-piperideine-6-carboxylate	43600	1074
		L-glutamate	520	13
		water	204	
(6*R*)-L-[6-^{3}H]lysine + L-[U-^{14}C]lysine	1083 (^{3}H) 3738 (^{14}C)[b]	Δ^1-piperideine-6-carboxylate	41470 (^{3}H) 132800 (^{14}C)[c]	1168 (^{3}H) 3741 (^{14}C)[c]
(6*S*)-L-[6-^{3}H]lysine	5720	Δ^1-piperideine-6-carboxylate	570	134
		L-glutamate	510	117
		water	31480	

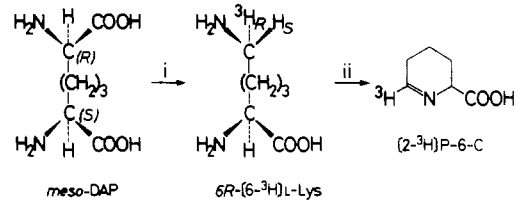
[a] Reactions and assays were carried out as described under Experimental Procedures. [b] ^{14}C/^{3}H ratio = 3.45. [c] ^{14}C/^{3}H ratio = 3.20.

Scheme II: Transamination between L-Lysine and α-Ketoglutarate Catalyzed by L-Lysine ε-Aminotransferase[a]



[a] Abbreviations: L-Lys, L-lysine; α-KG, α-ketoglutarate; α-AA-δ-SA, α-aminoadipic δ-semialdehyde; L-Glu, L-glutamate; P-6-C, Δ^1-piperideine-6-carboxylate. The asterisked hydrogen in α-AA-δ-SA is retained in P-6-C produced by the spontaneous cyclization of α-AA-δ-SA.

Scheme III: Conversion of *meso*-α,ε-Diaminopimelate to Δ^1-Piperideine-6-carboxylate (A)[a]

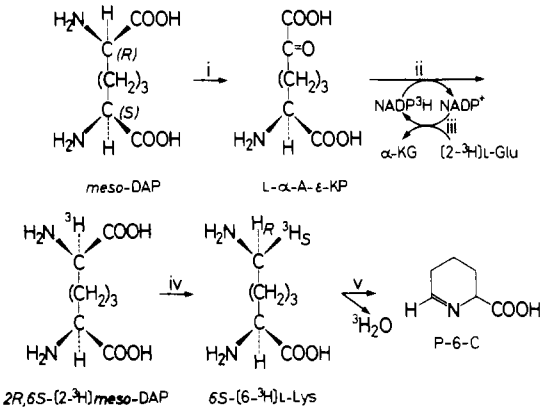


[a] Enzymes and reagents: (i) *meso*-DAP decarboxylase in ^3H_2O; (ii) L-lysine ε-aminotransferase and α-ketoglutarate.

samples of stereospecifically tritiated L-lysine. These samples were prepared from *meso*-DAP with *meso*-DAP decarboxylase, which acts on the carbon atom of *meso*-DAP with the D configuration to produce L-lysine. We recently have shown that this decarboxylation proceeds with inversion of configuration (Asada et al., 1981b). Thus, decarboxylation of *meso*-DAP in ^3H_2O afforded (6*R*)-L-[6-^{3}H]lysine (Scheme III). In contrast, (6*S*)-L-[6-^{3}H]lysine was synthesized by decarboxylation of (2*R*,6*S*)-*meso*-[2-^{3}H]DAP in water, which was prepared by reductive amination of L-α-amino-ε-ketopimelate in the presence of [4-^{3}H]NADPH with *meso*-DAP D-dehydrogenase (Scheme IV).

When (6*R*)-L-[6-^{3}H]lysine was used as a substrate for L-lysine ε-aminotransferase, most of the radioactivity was found in an isolated product, Δ^1-piperideine-6-carboxylate (Table I). In addition, no essential change in the radioactivity ratio (^{14}C/^{3}H) was observed between the substrate [L-[U-^{14}C]lysine plus (6*R*)-L-[6-^{3}H]lysine] and the product (Δ^1-piperideine-6-carboxylate). By contraries, almost all the radioactivity was released into the solvent, water, in the transamination between (6*S*)-L-[6-^{3}H]lysine and α-ketoglutarate. A significant amount of the radioactivity could not be detected in L-glutamate in

Scheme IV: Conversion of *meso*-α,ε-Diaminopimelate to Δ^1-Piperideine-6-carboxylate (B)[a]



[a] Enzymes and reagents: (i) *meso*-DAP D-dehydrogenase and NADP$^+$; (ii) *meso*-DAP D-dehydrogenase, NH_4Cl, and [4-^{3}H]-NADPH; (iii) glutamate dehydrogenase; (iv) *meso*-DAP decarboxylase in H_2O; (v) L-lysine ε-aminotransferase and α-ketoglutarate. L-α-A-ε-KP, L-α-amino-ε-ketopimelate.

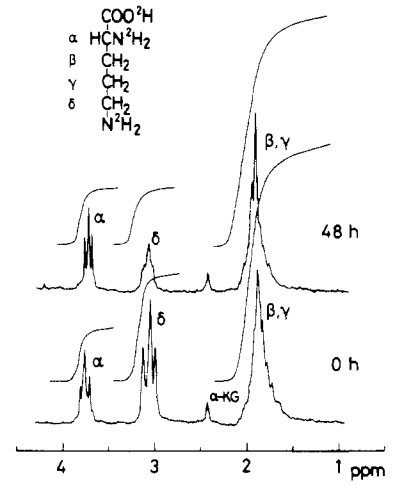
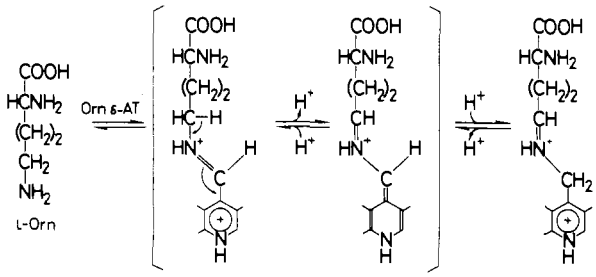


FIGURE 1: ^{1}H NMR spectral change observed during incubation of L-ornithine with L-ornithine δ-aminotransferase. The NMR tube contained 50 μmol of L-ornithine, 5 μmol of α-ketoglutarate (α-KG), and 100 μmol of potassium phosphate buffer (p^2H 8.0) in 1 mL of ^2H_2O. The spectrum was taken at 28 °C before (0 h) and after (48 h) addition of L-ornithine δ-aminotransferase (50 μg, 2.5 units). Incubation of the tube was carried out at 37 °C. Chemical shifts were referenced to sodium 3-(trimethylsilyl)propionate-d_4 at 0 ppm.

both reactions. These results indicate that L-lysine ε-aminotransferase discriminates two hydrogens at the prochiral C-6 carbon of L-lysine and abstracts the *pro-S* proton stereospecifically.

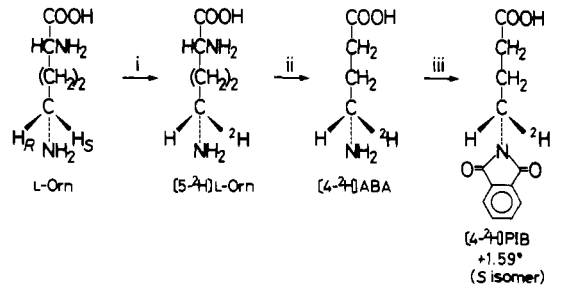
Stereospecificity for Proton Exchange Catalyzed by L-*Ornithine δ-Aminotransferase.* The initial process of the L-ornithine δ-aminotransferase reaction may be viewed as the reversible formation of the cofactor–substrate Schiff base

Scheme V: Initial Process of the L-Ornithine δ-Aminotransferase Reaction[a]



[a] Abbreviations: L-Orn, L-ornithine; Orn δ-AT, L-ornithine δ-aminotransferase.

Scheme VI: Conversion of L-Ornithine to 4-Phthalimido[4-^{2}H]butyrate[a]



[a] Enzymes and reagents: (i) L-ornithine δ-aminotransferase in ^2H_2O; (ii) L-lysine α-oxidase and H_2O_2; (iii) phthalic anhydride. The value below the structure is a specific rotation at 589 nm. L-Orn, L-ornithine; [4-^{2}H]ABA, 4-amino[4-^{2}H]butyrate; [4-^{2}H]PIB, 4-phthalimido[4-^{2}H]butyrate.

followed by abstraction of a proton from the prochiral C-5 carbon of L-ornithine (Scheme V). Figure 1 shows the ^{1}H NMR spectral change observed during incubation of L-ornithine with the enzyme in ^2H_2O. After incubation at 37 °C for 48 h, the peak integral of the C-5 protons was reduced to about half, whereas that of other protons did not change. This suggests that one of the two hydrogen atoms at C-5 is slowly exchanged with the solvent in the half-reaction of transamination. This proton exchange was promoted by the presence of a small amount of the amino acceptor, α-ketoglutarate [about one-tenth the amount (mole) of that of L-ornithine], although the role of α-ketoglutarate in the proton exchange remains unsettled.

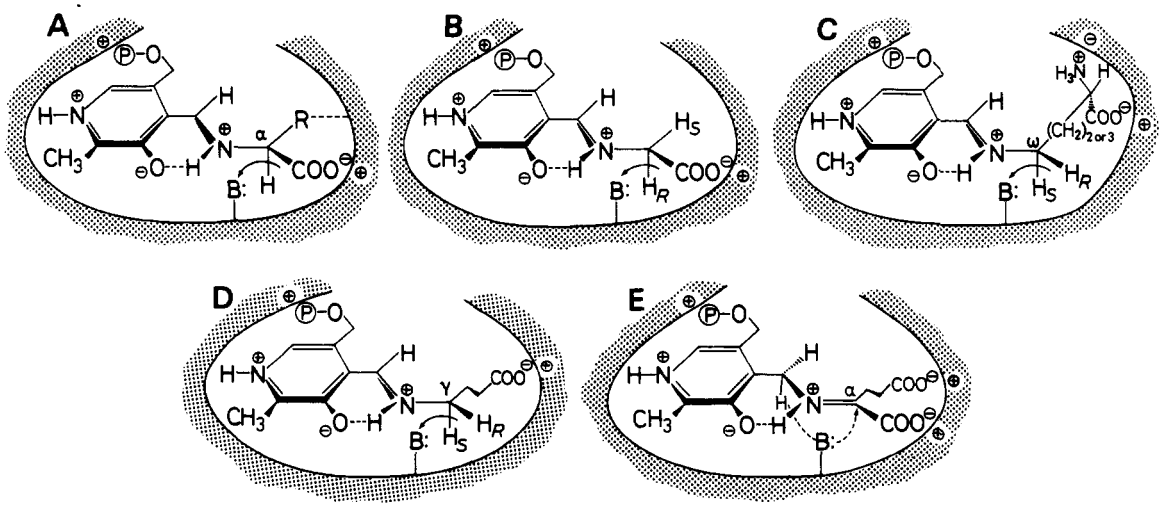
For determination of the absolute configuration of the C-5 carbon, L-[5-^{2}H]ornithine was converted to 4-phthalimido[4-^{2}H]butyrate by the procedure including oxidation by L-lysine α-oxidase, which acts also on L-ornithine (relative activity about 20% of that of L-lysine; Kusakabe et al., 1980). α-Keto-δ-aminovalerate produced from L-ornithine was successively decarboxylated by hydrogen peroxide to yield 4-amino[4-^{2}H]butyrate and finally converted to 4-phthalimido[4-^{2}H]butyrate (Scheme VI). This contained a single deuterium atom at the C-4 position and had an optical rotation of +1.59° at 589 nm (*c* 3.5, ethanol). Yamada & O'Leary (1978) reported in their stereochemical study of the L-glutamate decarboxylase reaction that (*R*)-4-phthalimido[4-^{2}H]butyrate had an optical rotation of −1.69°, i.e., a closely similar but opposite rotation to that derived from L-ornithine. Thus, the product from L-ornithine has the *S* configuration, and L-ornithine δ-aminotransferase exchanges the *pro-S* hydrogen at the prochiral C-5 carbon of L-ornithine with the solvent (see Scheme VI).

Discussion

Although stereochemical studies of various pyridoxal-P enzymes have led to a more detailed understanding of the dynamic aspects of catalysis by these enzymes as reviewed by Vederas & Floss (1980), only little attention has been paid to stereochemistry of enzymatic ω-transamination of diaminomonocarboxylic acids. We here showed that both bacterial L-lysine ε-aminotransferase and L-ornithine δ-aminotransferase withdraw exclusively the *pro-S* hydrogen from the prochiral ω-carbon atom of amino donors. Thus, the stereospecificity of the proton abstraction catalyzed by these ω-aminotransferases is the same as that reported for bacterial (Burnett et al., 1979) and mammalian (Bouclier et al., 1979) γ-aminobutyrate aminotransferases.

Scheme VII shows the probable orientation of the amino donors at the active sites of general L-amino acid α-aminotransferases (Scheme VIIA) and the above ω-aminotransferases (Scheme VIIC,D) along with that of glycine, whose *pro-R* hydrogen predominantly is abstracted by L-alanine aminotransferase (Hill, 1978) (Scheme VIIB). The C–H bonds to be broken are situated in the same stereochemical positions if the enzymes orient the substrates in the same direction by binding the amino group with the enzyme-bound pyridoxal-P and the carboxyl group or the side-chain moiety

Scheme VII: Schematic Representations of the Probable Orientation of Substrates at the Active Sites of α- and ω-Aminotransferases[a]



[a] (A) Amino donors at the active sites of L-amino acid α-aminotransferases; (B) glycine at the active site of L-alanine aminotransferase; (C) L-lysine and L-ornithine at the active sites of ω-aminotransferases; (D) γ-aminobutyrate at the active site of γ-aminobutyrate aminotransferase; (E) α-ketoglutarate at the active sites of L-lysine, L-ornithine, and γ-aminobutyrate ω-aminotransferases.

with the enzyme protein. Considering that the L(*S*) enantiomer of glutamate is produced from the amino acceptor, α-ketoglutarate, in these ω-aminotransferase reactions, the specificity for *pro-S* hydrogens may be stereochemically consequential on the assumption that α-ketoglutarate would occupy the same binding sites as those of amino donors (see Scheme VIIE). The ω-aminotransferases can, therefore, be regarded as L-amino acid α-aminotransferases on the basis of the reverse half-reaction from α-ketoglutarate to L-glutamate.

The stereochemical coincidence described above supports the Dunathan's hypothesis (Dunathan & Voet, 1974) that all pyridoxal-P enzymes have evolved from a common progenitor on the basis of the consistent stereochemistry of pyridoxal-P dependent enzyme reactions. However, ω-amino acid:pyruvate aminotransferase of *Pseudomonas* sp. F-126 is exceptional in this regard; it abstracts the *pro-R* hydrogen from the C-4 carbon of γ-aminobutyrate (Burnett et al., 1979).

As suggested by Dunathan (1971), the observed internal transfer of a labeled hydrogen from the amino acid substrate to the C-4′ carbon of pyridoxamine 5′-phosphate or from the cofactor to the α-keto acid substrate (Ayling et al., 1968; Bailey et al., 1970) and the ^{1}H NMR analyses of the L-alanine aminotransferase catalyzed proton exchange in ^2H_2O (Cooper, 1976; Golichowski et al., 1977) indicate a suprafacial cis transfer mediated by a single group of the enzyme functioning as a general acid–base catalyst. The catalytic base of any pyridoxal-P enzymes has not been elucidated. However, the lysine residue (Yamasaki et al., 1975) and histidyl residue (Peterson & Martinez-Carrion, 1970; Cheng & Martinez-Carrion, 1972; Martinez-Carrion et al., 1973; Giannini et al., 1975) have been suggested as the catalytic base of glutamate–aspartate aminotransferase. We here showed that the tritium labeled at the *pro-S* position of C-6 of L-lysine was little conserved in the product, L-glutamate, in the transamination but was liberated into the solvent. This suggests that the *S*-^{3}H is transferred to a group of the enzyme that rapidly exchanges the tritium with the solvent or to a polyprotic base such as the ε-amino group of lysine, resulting in the dilution of the label and thereby in the low efficiency of the tritium transfer to L-glutamate.

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