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Stereochemistry of *meso*-α,ϵ-Diaminopimelate Decarboxylase Reaction: The First Evidence for Pyridoxal 5′-Phosphate Dependent Decarboxylation with Inversion of Configuration†

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ABSTRACT: The stereochemistry of the decarboxylation of *meso*-α,ϵ-diaminopimelate catalyzed by *meso*-α,ϵ-diaminopimelate decarboxylase (EC 4.1.1.20) of *Bacillus sphaericus* was determined by stereochemical analyses of [6-^{2}H]-L-lysine produced by the reaction in D_2O. The product [6-^{2}H]-L-lysine was converted to levorotatory methyl 5-phthalimido[5-^{2}H]-valerate by the reactions not affecting the absolute configuration of the asymmetric carbon atom. By contrast, methyl 5-phthalimido[5-^{2}H]valerate derived from [2,6-$^2H_2$]-L-lysine, which was produced from [2,6-$^2H_2$]diaminopimelate by decarboxylation in H_2O, was dextrorotatory. The authentic methyl (*R*)-5-phthalimido[5-^{2}H]valerate prepared from L-glutamate with glutamate decarboxylase was levorotatory. These results indicate that the *meso*-α,ϵ-diaminopimelate decarboxylase reaction proceeds in an inversion mode. The deuterium label in [6-^{2}H]-L-lysine was fully conserved during the conversion into pelletierine through [1-^{2}H]cadaverine by the stereospecific diamine oxidase reaction. Thus, the enzymatic decarboxylation of *meso*-α,ϵ-diaminopimelate occurs with inversion of configuration in contrast to the other amino acid decarboxylases reported so far.

The reactions catalyzed by pyridoxal-P[1] dependent enzymes proceed through the reversible formation of a Schiff base between the 4′-aldehyde group of the cofactor and a primary amino group of a substrate (Snell & Di Mari, 1970). The following cleavage of one of the bonds to the substrate α carbon in the Schiff base yields a carbanionic quinoid intermediate. The specificity of this bond breaking as well as the nature of the reaction thereafter is determined by the structure of substrate and by the influence of the apoenzyme. Stereochemical analyses have provided a common feature of pyridoxal-P enzyme reactions as reviewed by Dunathan (1971). Thus, all bond-forming and bond-breaking reactions would occur on the same side of the plane formed with the Schiff base and the cofactor pyridine ring (Dunathan & Voet, 1974; Vederas & Floss, 1980). In support of this suggestion, all pyridoxal-P dependent decarboxylases studied so far catalyze

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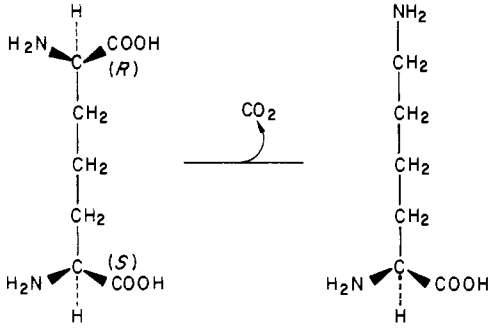
[1] Abbreviations used: pyridoxal-P, pyridoxal 5′-phosphate; DAP, α,ϵ-diaminopimelate; NMR, nuclear magnetic resonance.

0006-2960/81/0420-6881$01.25/0

decarboxylation with retention of configuration, i.e., L-tyrosine decarboxylase (Belleau & Burba, 1960; Battersby et al., 1980a), L-lysine decarboxylase (Leistner & Spenser, 1975; Gerdes & Leistner, 1979), L-glutamate decarboxylase (Yamada & O'Leary, 1978; Bouclier et al., 1979; Santaniello et al., 1979), and L-histidine decarboxylase (Battersby et al., 1979).

Recently, we have purified *meso*-DAP decarboxylase (EC 4.1.1.20) to homogeneity from the cell-free extract of *Bacillus sphaericus* and have reported briefly its enzymological properties (Asada et al., 1981). The enzyme participates in the final step of the bacterial lysine biosynthesis. This enzyme, requiring pyridoxal-P as a coenzyme, is the only known amino acid decarboxylase that acts on the carbon atom with D configuration in the substrate.



It is of particular interest to know the stereochemical course of the enzymatic decarboxylation of *meso*-DAP. In the present paper, we describe the stereochemical study of the *meso*-DAP decarboxylase reaction and provide the first proven instance of enzymatic amino acid decarboxylation with inversion of configuration.

Experimental Procedures

Materials. DAP was synthesized from glutaraldehyde according to the method of Roy & Karel (1973), and the DD, LL, and meso isomers of DAP were separated as described by Wade et al. (1957). *meso*-DAP decarboxylase of *Bacillus sphaericus* IFO 3525 was purified as reported previously (Asada et al., 1981). Amino acid racemase of low substrate specificity was prepared from cell extracts of *Pseudomonas putida* (formerly *Ps. striata*) IFO 12996 (Soda & Osumi, 1969). The homogeneous L-lysine α-oxidase purified from an aqueous extract of wheat bran culture of *Trichoderma viride* Y244-2 was kindly provided by Dr. H. Kusakabe, Yamasa Shoyu Co. (Kusakabe et al., 1980). L-Glutamate decarboxylase of *Escherichia coli* (type V) and L-lysine decarboxylase of *Bacterium cadaveris* (type VI) were purchased from Sigma. Diamine oxidase was extracted from pea seedlings and purified up to step 4 of the procedure given in the literature (Hill, 1971). D_2O (99.75 atom % D) was obtained from Merck. The other chemicals were analytical grade reagents.

General Instrumentation. Optical rotation was measured at 589 nm with a Perkin-Elmer 241 polarimeter with a 10-cm light path. ^1H NMR spectra were taken with a JEOL FX-100 recording spectrometer operated at 100 MHz. Sodium 3-(trimethylsilyl)propionate-*2,2,3,3-d*$_4$ and tetramethylsilan were used as an internal standard for the measurements in D_2O and in $CDCl_3$, respectively, and chemical shifts are reported in δ values (ppm). Mass spectra were obtained with a JEOL JMS-01SG mass spectrometer. Gas chromatography was performed with a Shimadzu GC-4BM gas chromatograph on a coiled glass column (3 mm × 2 m) packed with 5% OV-17 on Gas Chrom Q (100–200 mesh). Nitrogen was used as a carrier gas at a flow rate of 60 mL/min. Gas chromatography–mass spectrometry was carried out with a Shimadzu LKB-9000 gas chromatograph–mass spectrometer equipped with a Mas Pac-300 DGB computer data system. Ionization voltage, acceleration voltage, emission current, and slits were 70 eV, 3.5 kV, 60 μA, and 0.1 mm, respectively. The ion source was kept at 290 °C. Gas chromatography was run on a coiled glass column (3 mm × 1.5 m) stuffed with a mixture of 3% OV-17 and 1% OV-210 on Supelcoport (100–120 mesh). Helium was used as a carrier gas at a flow rate of 30 mL/min. The injection port and interface to the mass spectrometer were kept at 250 and 280 °C, respectively. Selective ion monitoring focused at m/e M − 2, M − 1, M^+, and M + 1 was conducted with a Shimadzu 9060S high-speed multiple ion detector–peak matcher equipment at an electron energy of 20 eV. Amino acids were determined by the method of Spackman et al. (1958) with a Hitachi 835 amino acid analyzer.

Preparation of [2,6-$^2H_2$]DAP. The reaction mixture (pD 7.4) containing 3 g of DAP (a mixture of DD, LL, and meso isomers) and 4.2 mg of amino acid racemase (5000 units) in 50 mL of D_2O was incubated at 30 °C for 24 h with stirring. After the reaction was stopped by addition of 50 mL of ethanol (99.5 vol %), the precipitated protein was removed by centrifugation. The supernatant solution was evaporated to dryness under reduced pressure, and exchangeable deuterium atoms in the residue were removed by repeated evaporations and dissolutions in 20 mL of water. The recovery of DAP determined with an amino acid analyzer was about 97% (2.9 g). The ^1H NMR spectrum of the product in D_2O showed 99.0 atom % excess deuterium at the C-2 and C-6 positions (δ 3.5) of DAP.

Preparation of [6-^2H]-L-Lysine. meso-DAP decarboxylase (3.6 mg, 100 units) was dissolved in D_2O, and H_2O was removed by repeated concentrations with an Amicon 202 ultrafiltration unit and dilutions with 10 mM potassium phosphate buffer in D_2O (pD 6.8) containing 0.1 mM pyridoxal-P and 0.01% 2-mercaptoethanol. The solution containing 700 mg of *meso*-DAP, 2.65 mg of pyridoxal-P, and 16 mmol of potassium phosphate buffer (pD 6.8) also was freed from exchangeable protons by repeated evaporations and dissolutions in D_2O and adjusted to a final volume of 100 mL with D_2O. The reaction was started by addition of the enzyme solution (2 mL) and carried out at 37 °C for 12 h. After addition of 9.1 mL of 6 N HCl and centrifugation, the supernatant solution was applied to a Dowex 50-X8 (H^+ form) column (2 × 15 cm). The column was washed with 200 mL of 1.7 N HCl to remove the unreacted *meso*-DAP, and [6-^{2}H]-L-lysine was eluted with 200 mL of 2.0 N HCl in 5-mL fractions. The fractions positive to ninhydrin test were pooled and evaporated to dryness. The purity of the product was confirmed by amino acid analysis and thin-layer chromatography on a Tokyo Kasei S205 cellulose film with a solvent system of 1-butanol–acetic acid–water (4:1:2 v/v/v) (R_f 0.18). The yield determined by amino acid analysis was about 400 mg (74%).

Preparation of [2,6-$^2H_2$]-L-Lysine. The reaction mixture containing 5.4 mg of *meso*-DAP decarboxylase (150 units), 1.2 g of [2,6-$^2H_2$]DAP, (a mixture of DD, LL, and meso isomers), 0.5 mmol of potassium phosphate buffer (pH 6.8), 5 μmol of pyridoxal-P, and 125 μmol of 2,3-dimercaptopropan-1-ol in a final volume of 50 mL was incubated at 37 °C for 12 h. [2,6-$^2H_2$]-L-Lysine produced was isolated and identified as described above. The yield was 340 mg (37%).

Conversion of [6-^2H]-L-Lysine and [2,6-$^2H_2$]-L-Lysine into Methyl 5-Phthalimido[5-^2H]valerate. Both [6-^{2}H]-L-lysine and [2,6-$^2H_2$]-L-lysine were first converted to the labeled

5-aminovaleric acid by the action of L-lysine α-oxidase as follows: It catalyzes the oxidation of L-lysine to hydrogen peroxide and α-keto-ε-aminocaproic acid. The latter is successively decarboxylated by hydrogen peroxide to yield 5-aminovaleric acid (Kusakabe et al., 1980). To 100 mL of 10 mM potassium phosphate buffer (pH 8.0) containing 400 mg of [6-^{2}H]-L-lysine or 340 mg of [2,6-$^2H_2$]-L-lysine was added 2.1 mg of L-lysine α-oxidase (140 units), and the mixture was incubated at 37 °C for 12 h with stirring. The reaction was terminated by addition of 9.1 mL of 6 N HCl, and the denatured enzyme was removed by centrifugation. The supernatant solution was chromatographed on a Dowex 50-X8 (H^+ form) column (2 × 15 cm) equilibrated with 1.0 N HCl. After the column was washed with 1.0 N HCl, 5-amino[5-^{2}H]valeric acid was eluted with 1.5 N HCl, and the eluate was evaporated to dryness. The product was determined by amino acid analysis and thin-layer chromatography with the same system as described above (R_f 0.55). The yield of 5-amino[5-^{2}H]-valeric acid was 110 mg (34%) from [6-^{2}H]-L-lysine and 100 mg (37%) from [2,6-$^2H_2$]-L-lysine.

About 100 mg of 5-amino[5-^{2}H]valeric acid was then converted to 5-phthalimido[5-^{2}H]valeric acid by refluxing with 120 mg of phthalic anhydride in 3 mL of toluene and 0.15 mL of triethylamine under a Liensterg-type water separator for 2 h (Bose, 1960). The product was crystallized from aqueous ethanol (yield 103 mg, 49%), and it gave a single spot upon thin-layer chromatography on a Merck 60F_{254} silica gel plate with chloroform–methanol (20:1 v/v) as a solvent (R_f 0.68). Peak integrals of ^{1}H NMR spectra of the products in $CDCl_3$ showed the quantitative incorporation of one deuterium atom into the C-5 position (δ 3.7) of 5-phthalimido[5-^{2}H]valeric acid derived from both [6-^{2}H]-L-lysine and [2,6-$^2H_2$]-L-lysine.

The two samples of 5-phthalimido[5-^{2}H]valeric acid (100 mg each) were finally esterified with diazomethane in ether. Methyl 5-phthalimido[5-^{2}H]valerate was identified by ^{1}H NMR, thin-layer chromatography with the same system as described above (R_f 0.92), and gas chromatography (retention time 16 min, 180–270 °C, 5 °C/min) and submitted for the measurement of optical rotation.

Preparation of Methyl (R)-(−)-5-Phthalimido[5-^{2}H]valerate. The levorotatory methyl 5-phthalimido[5-^{2}H]valerate was prepared from (*R*)-(−)-4-amino[4-^{2}H]butyric acid, which was synthesized according to the method of Yamada & O'Leary (1978) with the following modifications. The glutamate decarboxylase reaction was carried out at 37 °C for 24 h with stirring in a reaction mixture containing 2.5 g of L-glutamic acid, 2.5 g of sodium L-glutamate, 50 mg of pyridoxal-P, 25 mg of dithiothreitol, 6.7 mg of L-glutamate decarboxylase (150 units), and 5 mmol of pyridine–DCl buffer (pD 4.5) in 50 mL of D_2O. Prior to the reaction, exchangeable protons were removed from all the reagents by repeated evaporations and dissolutions in D_2O. After addition of 10 mL of 50% trichloroacetic acid and centrifugation, the supernatant solution was adjusted to pH 4.0 with pyridine and chromatographed on Dowex 50-X8 (pyridinium form, 2.6 × 35 cm) equilibrated with 10 mM pyridine–acetate buffer (pH 4.0). Unreacted L-glutamic acid was washed out with the buffer, and (*R*)-(−)-4-amino[4-^{2}H]butyric acid was eluted with 10 mM pyridine–acetate buffer (pH 5.5). The product was crystallized from ethanol–water (yield 1.1 g) and identified by amino acid analysis, mass spectrometry, and elemental analysis. Anal. Calcd for 4-aminobutyrate ($C_4H_8DNO_2$): C, 46.2; H + D, 9.6; N, 13.5. Found: C, 46.4; H + D, 9.1; N, 13.4. The ^{1}H NMR and mass spectral analyses indicated the presence of one deuterium atom at the C-4 position (δ 3.0) of the product.

After about 300 mg of (*R*)-(−)-4-amino[4-^{2}H]butyric acid was converted to (*R*)-(−)-4-phthalimido[4-^{2}H]butyric acid by the same procedure as described above, the phthalimide derivative (190 mg) was treated successively with $SOCl_2$, diazomethane, and Ag_2O in methanol as reported by Yamada & O'Leary (1978) to yield methyl (*R*)-(−)-5-phthalimido[5-^{2}H]valerate. The product was purified by preparative-scale thin-layer chromatography on a Merck 60F_{254} silica gel plate (thickness 2.0 mm) with chloroform–methanol (100:1 v/v) as a solvent and identified by mass spectrometry and gas chromatography. The peak integral of the ^{1}H NMR spectrum indicated the presence of one deuterium atom at the C-5 position (δ 3.7) of the product.

Preparation of [1-^{2}H]Cadaverine. The reaction mixture containing 170 mg of [6-^{2}H]-L-lysine and 10 mg of L-lysine decarboxylase (26 units) in a volume of 60 mL (pH 5.8 adjusted with 2 N acetic acid) was incubated at 30 °C for 12 h with stirring. After heating at 100 °C for 5 min and centrifugation, the supernatant solution was evaporated to dryness. The product [1-^{2}H]cadaverine was identified by paper chromatography on a Toyo No. 51 filter paper with 2-propanol–ammonia (28%)–water (8:1:1 v/v/v) as a solvent (R_f 0.5).

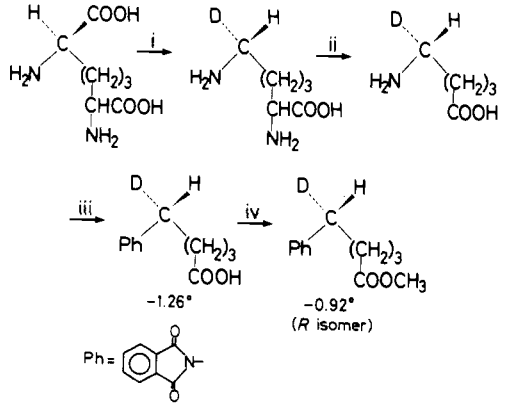
The deuterium content of [1-^{2}H]cadaverine was examined by gas chromatography–mass spectrometry as follows. To a portion of the residue was added 0.6 mL of an acetonitrile solution of *N*,*N*-dimethylformamide dimethyl acetal (1:1 v/v), which reacts with amino groups of cadaverine to form *N*,*N*′-bis[(dimethylamino)methylene]cadaverine (Thenot & Horning, 1972). The reaction was carried out in a microvial with a Teflon cap liner at 80 °C for 10 min. The reaction mixture was subjected directly to gas chromatographic–mass spectrometric analysis. The column was programmed isothermally at 180 °C. The deuterated derivative, whose retention time was 3 min under the conditions used, was identical by gas chromatography with the same derivative of authentic unlabeled cadaverine. The deuterium content was calculated from the data obtained with a mass spectrometer equipped with a selective ion monitor according to the method of Biemann (1962).

Preparation of Pelletierine. [1-^{2}H]Cadaverine prepared above was converted to pelletierine by diamine oxidase as reported previously (Gerdes & Leistner, 1979) with the following modifications. The reaction mixture containing 2.5 mg of [1-^{2}H]cadaverine, 0.4 mg of diamine oxidase (15 units), 30 mg of lithium acetoacetate, and 50 μmol of potassium phosphate buffer (pH 7.5) in a total volume of 1 mL was incubated at 37 °C for 12 h, followed by addition of 0.2 mL of 6 N HCl and heating at 100 °C for 10 min. After the removal of the precipitated protein by centrifugation, the pH of the solution was adjusted to about 10 with 1 N KOH. Pelletierine extracted with ether was identical by thin-layer chromatography on a Merck 60F_{254} silica gel plate with dichloromethane–methanol–ammonia (28%) (50:20:3 v/v/v) as a solvent (R_f 0.8) to the authentic compound synthesized as described above from the unlabeled cadaverine. The ether solution of pelletierine was subjected to gas chromatographic–mass spectrometric analysis. The column of gas chromatography was operated isothermally at 100 °C. The deuterium content of the labeled pelletierine, whose retention time was 3 min under the conditions used, was calculated as above from mass spectrometric data.

Results

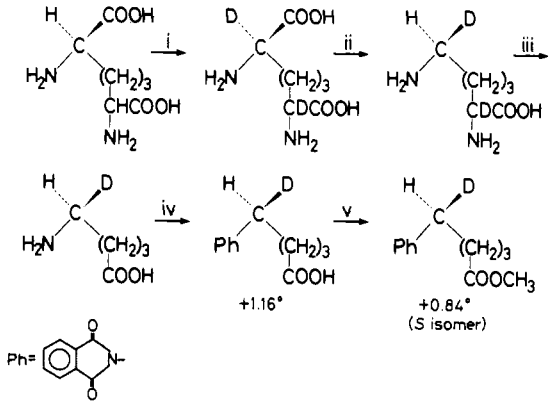
When *meso*-DAP was decarboxylated by *meso*-DAP decarboxylase of *Bacillus sphaericus* in D_2O, L-lysine produced was found to contain one deuterium atom at the C-6 position

Scheme I: Conversion of *meso*-DAP to Levorotatory Methyl 5-Phthalimido[5-^{2}H]valerate[a]



[a] Values below the structures are specific rotations at 589 nm. Enzymes and reagents: (i) *meso*-DAP decarboxylase in D_2O; (ii) L-lysine α-oxidase; (iii) phthalic anhydride; (iv) diazomethane.

Scheme II: Conversion of *meso*-DAP to Dextrorotatory Methyl 5-Phthalimido[5-^{2}H]valerate[a]



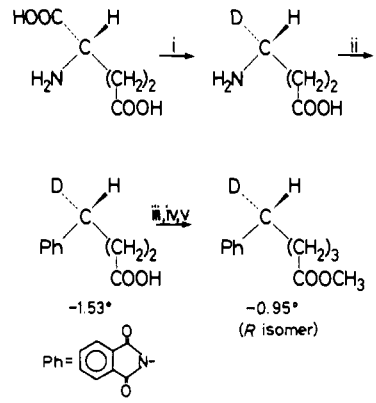
[a] Values below the structures are specific rotations at 589 nm. Enzymes and reagents: (i) amino acid racemase in D_2O; (ii) *meso*-DAP decarboxylase in H_2O; (iii) L-lysine α-oxidase; (iv) phthalic anhydride; (v) diazomethane.

(δ 3.0) by ^{1}H NMR analysis. For determination of the absolute configuration of the C-6 carbon, [6-^{2}H]-L-lysine was converted to methyl 5-phthalimido[5-^{2}H]valerate (Scheme I) by the similar procedure to that reported for the analysis of glutamate decarboxylase reaction (Yamada & O'Leary, 1978) except that the L-lysine α-oxidase reaction was included. None of the reactions employed in this conversion would affect the absolute configuration of the asymmetric carbon atom. Thus, the absolute configuration of the methyl ester would coincide with that of the starting [6-^{2}H]-L-lysine. The presence of one deuterium atom in the C-5 position of the methyl ester shown by ^{1}H NMR analysis also confirmed that no loss of the deuterium atom occurred during the conversion. Methyl 5-phthalimido[5-^{2}H]valerate thus obtained had an optical rotation of −0.92° at 589 nm (*c* 5.43, ethanol).

By contrast, methyl 5-phthalimido[5-^{2}H]valerate obtained from [2,6-$^2H_2$]-L-lysine, which was produced from [2,6-$^2H_2$]DAP by the decarboxylation in H_2O (Scheme II), had an optical rotation of +0.84° at 589 nm (*c*, 2.25, ethanol).

Methyl 5-phthalimido[5-^{2}H]valerate with the known configuration of the C-5 carbon atom was synthesized from the phthalimide derivative of (*R*)-4-amino[4-^{2}H]butyric acid by reactions for chain elongation of one carbon atom and simultaneous esterification, which cause no influence on the absolute configuration of the asymmetric carbon atom. (*R*)-4-Amino[4-^{2}H]butyric acid, whose optical rotation at 589 nm was determined to be −0.40° (*c* 4.47, 6 N HCl), in agreement with the reported value (−0.33°; Yamada & O'Leary, 1978), was prepared from L-glutamic acid by the established stereospecific decarboxylation catalyzed by L-glutamate decarboxylase in D_2O (retention) (Yamada & O'Leary, 1978). The measurement of the optical rotation of methyl (*R*)-5-phthalimido[5-^{2}H]valerate gave a value of −0.95° (*c* 2.75, ethanol).

Scheme III: Synthesis of Methyl (*R*)-5-Phthalimido[5-^{2}H]valerate from L-Glutamate[a]



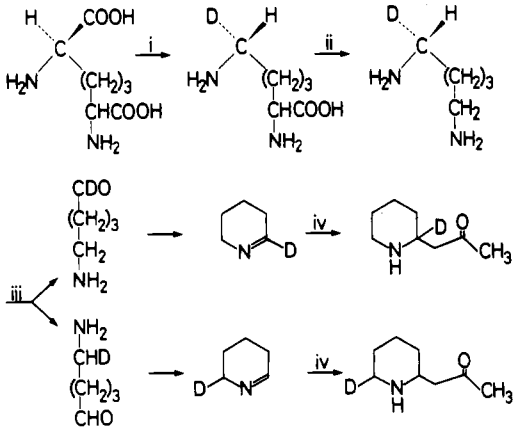
[a] Values below the structures are specific rotations at 589 nm. Enzymes and reagents: (i) L-glutamate decarboxylase in D_2O; (ii) phthalic anhydride; (iii) $SOCl_2$; (iv) diazomethane; (v) Ag_2O/CH_3OH.

As shown in Scheme III, the authentic methyl 5-phthalimido[5-^{2}H]valerate prepared from L-glutamic acid has the *R* configuration. The product from *meso*-DAP decarboxylated in D_2O has an identical rotation with that of the authentic compound whereas the complementary product from [2,6-$^2H_2$]DAP decarboxylated in H_2O has a nearly equal but opposite rotation. Hence, it is evident that the former product has the *R* configuration and the latter has the *S* configuration. *meso*-DAP decarboxylase acts stereospecifically on the carbon atom with D configuration (*R*) in the substrate (Asada et al., 1981). Therefore, these results indicate unequivocally that the decarboxylation of *meso*-DAP proceeds with inversion of configuration (Schemes I and II).

The results obtained above were in quite contrast with stereochemistry reported for other L-amino acid decarboxylase reactions, all of which take place with retention of configuration. Therefore, we examined the *meso*-DAP decarboxylase reaction in an another way. The [6-^{2}H]-L-lysine produced from *meso*-DAP by decarboxylation in D_2O was converted to [1-^{2}H]cadaverine by the action of L-lysine decarboxylase of *Bacterium cadaveris*. Diamine oxidase from pea seedlings was reported to catalyze the stereospecific removal of the *pro-S* hydrogen atom from the methylene carbon adjacent to terminal amino groups of cadaverine during oxidative deamination and yield 5-aminopentanal (Gerdes & Leistner, 1979). This diamine oxidase reaction had been employed for the stereochemical analysis of the L-lysine decarboxylase reaction by the measurement of isotope contents in pelletierine converted from cadaverine (Gerdes & Leistner, 1979).

Scheme IV illustrates the procedure used in the present study. The cadaverine molecule is symmetrical, and diamine oxidase labilizes the *pro-S* hydrogen of only one terminal methylene group of cadaverine. Thus, 50% loss of the labeled deuterium would be observed if the *meso*-DAP decarboxylase reaction proceeds with retention of configuration; i.e., the *pro-S* hydrogen of the C-6 position of L-lysine is deuterized. If the enzymatic decarboxylation of *meso*-DAP takes place with inversion of configuration, the deuterium atom (in the *R*

Scheme IV: Conversion of *meso*-DAP to Pelletierine[a]



[a] Enzymes and reagents: (i) *meso*-DAP decarboxylase in D_2O; (ii) L-lysine decarboxylase; (iii) diamine oxidase; (iv) acetoacetate.

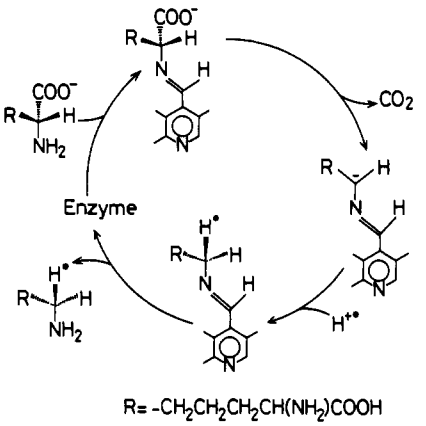
configuration) would be fully retained in the resulting pelletierine. Gas chromatographic–mass spectrometric analyses of the products obtained here showed that pelletierine contained essentially the same enrichment in deuterium (93.1% excess as an average value of 5 times measurements of mass spectrometry) as in [1-^{2}H]cadaverine (93.7% excess); the diamine oxidase reaction resulted in 99.4% retention of the deuterium label. Therefore, it was confirmed again that *meso*-DAP decarboxylase catalyzes the decarboxylation of *meso*-DAP with inversion of configuration.

Discussion

According to the Dunathan's hypothesis on the reaction mechanism of pyridoxal-P dependent enzymes, the bond to be broken in the substrate–pyridoxal-P complex aligns orthogonally to the plane of the extended conjugated π system (Dunathan, 1966). Thus, amino acid decarboxylases orient the substrate–pyridoxal-P complex in such a way that the α-carboxyl group is positioned perpendicularly to the conjugated system. The anionic intermediate formed after decarboxylation captures a proton to yield the product, an amine. Stereochemical analyses of amino acid decarboxylase reactions have shown that the protonation occurs only from one side of the planar imine (the same side as the carboxyl group liberates); the reactions by L-tyrosine (Belleau & Burba, 1960; Battersby et al., 1980a), L-lysine (Leistner & Spenser, 1975; Gerdes & Leistner, 1979), L-glutamate (Yamada & O'Leary, 1978; Bouclier et al., 1979; Santaniello et al., 1979), and L-histidine[2] (Battersby et al., 1979) decarboxylases proceed exclusively with retention of configuration.[3] This stereochemical consistency conforms to the suggestion by Dunathan & Voet (1974); all bond-making and bond-breaking processes in pyridoxal-P dependent enzyme reactions occur on the same face of the substrate–coenzyme complex with the exception of racemization.

We here present with *meso*-DAP decarboxylase the first evidence for the enzymatic amino acid decarboxylation in an inversion mode (Scheme V). The magnitude of the optical rotation of methyl 5-phthalimido[5-^{2}H]valerate and the

Scheme V: Stereochemical Course of *meso*-DAP Decarboxylase Reaction



R= -$CH_2CH_2CH_2CH(NH_2)COOH$

quantitative preservation of the deuterium labels show that the reaction is strictly stereospecific. Thus, it is highly unlikely that the protonation to the anionic intermediate of the *meso*-DAP–pyridoxal-P complex occurs directly from the solvent without the mediation of some proton donor of the enzyme, although the base involved in the proton transfer has not been elucidated.

There are two possible explanations for the inversion of configuration during the catalytic process of *meso*-DAP decarboxylase. First, the catalytic group which donates a proton to the α-carbon atom of the anionic intermediate exists on the opposite side of the plane to that of decarboxylation; this is based on the assumption that the enzyme binds with the pyridine nitrogen, the phosphate, and the distal group(s) of the substrate moiety to fix the substrate–pyridoxal-P complex rigidly and that no significant conformational change occurs during catalysis. As reported previously (Asada et al., 1981), the enzyme shows a strict substrate specificity to the meso form of DAP; the DD and LL enantiomers of DAP are neither substrates nor inhibitors. This suggests that the distal group(s) of the substrate plays (play) a role in the substrate binding to the active site of enzyme.

Secondly, a drastic conformational change leading to the rotation of the Schiff base plane and thereby to the protonation from the same direction as decarboxylation may occur after the initial decarboxylation step. Conformational changes that occur upon the substrate binding have been suggested for aspartate aminotransferase (Metzler et al., 1978; Gehring & Christen, 1978), tyrosine decarboxylase (Vederas et al., 1979), and tryptophanase (Vederas & Floss, 1980). However, a nearly 180° rotation after the formation of the anionic intermediate seems improbable because of necessity of an extremely large structural rearrangement.

Although our finding on the *meso*-DAP decarboxylation is incompatible with the current concept that pyridoxal-P enzyme reactions generally take place on only one side of the enzyme-bound substrate–cofactor complex (Vederas & Floss, 1980), the exceptional stereochemistry of *meso*-DAP decarboxylase may be a reflection of the fact that the enzyme is distinct from the other amino acid decarboxylases because of its specificity to the carbon atom with D configuration in the substrate. The stereospecific reduction of the Schiff base complex of *meso*-DAP decarboxylase with sodium boro-[^{3}H]hydride would give us some information. This strategy has been used for conformational analyses of Schiff base complexes of tryptophanase (Vederas et al., 1978), tyrosine decarboxylase (Vederas et al., 1979), and aspartate aminotransferase (Zito & Martinez-Carrion, 1980). Stereochemical analysis of the reaction of L-lysine:α-ketoglutarate ϵ-amino-

[2] The decarboxylation of L-histidine catalyzed by bacterial histidine decarboxylase (e.g., from *Lactobacillus* 30a), which does not require pyridoxal-P as a coenzyme but contains a pyruvoyl residue in the active site of enzyme (Recsei & Snell, 1970), also takes place with retention of configuration (Chang & Snell, 1968; Battersby et al., 1980b).

[3] When this paper was returned for revision, one of the reviewers offered recent information that *S*-adenosylmethionine decarboxylase of *Escherichia coli*, a pyruvate-containing amino acid decarboxylase, operates via a retentive mode (Allen & Klinman, 1981).

transferase based on the *meso*-DAP decarboxylase reaction is currently in progress.

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Nuclear Deoxyribonucleic Acid Characterization of the Marine Chromophyte *Olisthodiscus luteus*†

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ABSTRACT: Nuclear DNA of the marine chromophytic alga *Olisthodiscus luteus* was analyzed in this study. Reassociation kinetics analysis has shown that 440-nucleotide DNA fragments from the genome of this alga contain 4% foldback, 58% repetitive, and 34% single-copy sequences. Precise analysis using isolated single-copy DNA revealed that *Olisthodiscus* has a large haploid DNA content of 1.66×10^{-12} g/cell. For determination of the organization of single-copy and repetitive sequences within this genome, DNA fragments 3000 nucleotides in length were reassociated to C_0t = 100 M·s. At this low C_0t value 89% of the DNA bound to hydroxylapatite, suggesting that single-copy and repetitive elements are interspersed. The lengths of the duplexed repetitive DNA on these 3000-nucleotide fragments were measured by electron microscopy after digestion with S1 nuclease which removed the unreassociated single-copy DNA regions. Of these repetitive sequences, 68% were shorter than 1200 nucleotide pairs in length and had a modal length of 350 nucleotide pairs. A minor class of longer (to 4000 nucleotide pairs) repetitive sequences was also observed.

The Chromophyta represent a major evolutionary group of plants which have only minimal representation in the vast literature concerning eukaryotic nuclear morphology and biochemistry. Members of this taxonomic sequence include the Dinophyceae (dinoflagellates), Bacillariophyceae (diatoms), Chrysophyceae, and Chloromonadophyceae and culminate in the Phaeophyceae (brown algae including the kelps). The Chromophyta (chlorophyll *a,c* line) are thought to have split or to have arisen independently early in evolutionary time (Taylor, 1978) from those plants designated the Chlorophyta (chlorophyll *a,b* line). Unlike the Chromophyta, which only include algal species, the Chlorophyta include algae, ferns, and

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0006-2960/81/0420-6886$01.25/0