Fischer, H., & Mueller, A. (1937) *Hoppe-Seyler's Z. Physiol. Chem. 246*, 43–58.
Graden, D. W., & Lynn, D. G. (1984) *J. Am. Chem. Soc. 106*, 1119–1121.
Groves, J. T., Haushalter, R. C., Nakamura, M., Nemo, T. E., & Evans, B. J. (1981) *J. Am. Chem. Soc. 103*, 2884–2886.
Guengerich, F. P. (1978) *Biochemistry 17*, 3633–3639.
Guengerich, F. P., & Strickland, T. W. (1977) *Mol. Pharmacol. 13*, 993–1004.
Guengerich, F. P., Dannan, G. A., Wright, S. T., Martin, M. V., & Kaminsky, L. S. (1982) *Biochemistry 21*, 6019–6030.
Levin, W., Lu, A. Y. H., Jacobson, M., & Kuntzman, R. (1973) *Arch. Biochem. Biophys. 158*, 842–852.
Lightner, D. A., & Quistad, G. B. (1972) *FEBS Lett. 25*, 94–96.
Lowry, O. H., Rosebrough, N. J., Farr, A. L., & Randall, R. J. (1951) *J. Biol. Chem. 193*, 265–275.
Mansuy, D., Bartoli, J. F., & Momenteau, M. (1982) *Tetrahedron Lett. 23*, 2781–2784.
Nishibayashi, H., Omura, T., Sato, R., & Estabrook, R. W. (1968) in *Structure and Function of Cytochromes*, p 658, University Park Press, Baltimore, MD.
Omura, T., & Sato, R. (1964) *J. Biol. Chem. 239*, 2370–2378.
Ortiz de Montellano, P. R., & Correia, M. A. (1983) *Annu. Rev. Pharmacol. Toxicol. 23*, 481–503.
Rüdiger, W. (1970) *Angew. Chem. 9*, 473–480.
Sanders, J. K. M., Waterton, J. C., & Denniss, I. S. (1978) *J. Chem. Soc., Perkin Trans. 1*, 1150–1157.
Svingen, B. A., O'Neal, F. O., & Aust, S. D. (1978) *Photochem. Photobiol. 28*, 803–809.
Tenhunen, R., Marver, H. S., & Schmid, R. (1969) *J. Biol. Chem. 244*, 6388–6394.
van der Hoeven, T. A., & Coon, M. J. (1974) *J. Biol. Chem. 249*, 6302–6310.
Wiseman, J. S., Nichols, J. S., & Kolpak, M. X. (1982) *J. Biol. Chem. 257*, 6328–6332.
Yoshinaga, T., Sassa, S., & Kappas, A. (1982) *J. Biol. Chem. 257*, 7794–7802.

Stereochemistry of Lysine Formation by *meso*-Diaminopimelate Decarboxylase from Wheat Germ: Use of ^1H–^{13}C NMR Shift Correlation To Detect Stereospecific Deuterium Labeling†

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ABSTRACT: The stereochemical course of the wheat germ *meso*-diaminopimelate (DAP) decarboxylase reaction is compared to that of the decarboxylase isolated from *Bacillus sphaericus*, which has been reported to proceed with an unusual inversion of configuration [Asada, Y., Tanizawa, K., Sawada, S., Suzuki, T., Misono, H., & Soda, K. (1981) *Biochemistry 20*, 6881–6886]. Reaction of each enzyme with either unlabeled diaminopimelic acid in D_2O or [2,6-$^2H_2$]diaminopimelic acid in H_2O gave stereospecifically deuterium-labeled lysine samples that were derivatized with (−)-camphanoyl chloride and diazomethane. Analysis by two-dimensional ^1H–^{13}C heteronuclear NMR shift correlation spectroscopy with ^2H decoupling confirmed the stereochemistry of the *B. sphaericus* enzyme reaction and showed that the eukaryotic wheat germ *meso*-DAP decarboxylase also operates with inversion of configuration. This suggests similar mechanisms for the prokaryotic and eukaryotic enzymes and contrasts the retention mode observed with other pyridoxal phosphate dependent α-decarboxylases.

The enzyme *meso*-diaminopimelate (DAP)[1] decarboxylase (EC 4.1.1.20) is pyridoxal 5′-phosphate (PLP) dependent and catalyzes the decarboxylation of the R_D center of *meso*-diaminopimelic acid to give L-lysine (Figure 1) (Patte, 1983; White, 1983). This enzyme has been isolated from both plants (Vogel & Hirvonen, 1971; Mazelis & Creveling, 1978; Sodek, 1978; Kato, 1979; Rosenthal, 1982) and bacteria (White, 1971; Rosner, 1975; Asada et al., 1981a; Lakshman et al., 1981) and is the only PLP-dependent α-decarboxylase known to act on a D-amino acid. The enzyme isolated from *Bacillus sphaericus* has been reported by Soda and co-workers (Asada et al., 1981b) to operate with inversion of configuration, in direct contrast to other PLP-dependent α-decarboxylases for which the stereochemistry of reaction has been determined. In all other cases examined, the reaction proceeds with retention of configuration—that is, the incoming proton occupies the same stereochemical position as did the departing carboxyl group (Dunathan, 1971; Snell, 1982; Floss & Vederas, 1982; Akhtar et al., 1984; Palcic & Floss, 1985; Nakazawa et al., 1981; Orr

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[1] Abbreviations: PLP, pyridoxal 5′-phosphate; DAP, diaminopimelate; DTE, dithioerythritol; EtOH, ethanol; EtOAc, ethyl acetate; EDTA, ethylenediaminetetraacetate; Me_4Si, tetramethylsilane.

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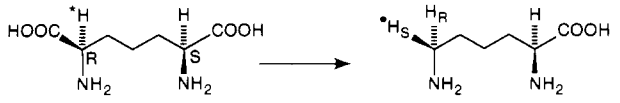


FIGURE 1: Reaction catalyzed by *meso*-DAP decarboxylase.

& Gould, 1982; Orr et al., 1984; Wigle et al., 1982; Robins, 1983; Asada et al., 1984). In view of the observation that many other types of PLP enzymatic reactions occur on a single face of the substrate–cofactor complex [Liu et al. (1984) and references cited therein], it appeared important to confirm the unique *B. sphaericus* decarboxylase results and to determine whether the corresponding enzyme from eukaryotic sources like wheat germ behaves similarly.

Determination of the stereochemical course of PLP-dependent decarboxylase catalyzed reactions ultimately involves the analysis of the configuration of a methylene group bearing two different isotopes of hydrogen. This has been accomplished by many methods [Floss & Vederas, 1982; Akhtar et al., 1984; Palcic & Floss (1985) and references cited therein], most of which involve correlation of the decarboxylation product with a material of known stereochemistry by a series of enzymatic or chemical reactions. NMR methods have also been employed; both direct ^1H NMR (Nakazawa et al., 1981) and ^1H NMR of camphanamide derivatives of the product amines with europium shift reagents (Santaniello et al., 1979; Orr & Gould, 1982; Orr et al., 1984) may permit configurational assignment. Recently, in our laboratory, a new NMR technique for observing the position of isotopic hydrogen in stereospecifically deuterium-labeled methylene groups has been developed (Trimble et al., 1985). Either selective or normal two-dimensional ^1H–^{13}C heteronuclear NMR shift correlation spectroscopy (Levitt et al., 1983; Pegg & Bendall, 1983; Nakashima et al., 1984a,b) in combination with ^2H decoupling easily resolves overlapping resonances and allows facile identification of deuterium-labeled hydrogens with good sensitivity. The technique is applied here to determine the stereochemical course of both the *B. sphaericus* and the wheat germ *meso*-DAP decarboxylase reactions.

Experimental Procedures

Materials. All chemicals were obtained from Sigma except ACS scintillation cocktail and [1,7-^{14}C]diaminopimelate (sp. act. 118 mCi/mmol) (Amersham); (−)-camphanic acid chloride was from Aldrich; AG 50W-X8 cation-exchange resin, D_2O (99.8 atom % D), and protein assay reagents were from Bio-Rad. Alcohol-free ethereal diazomethane was prepared from Diazald (*N*-methyl-*N*-nitroso-*p*-toluenesulfonamide) with the Aldrich diazomethane generator kit and the procedure outlined on the Diazald bottle label.

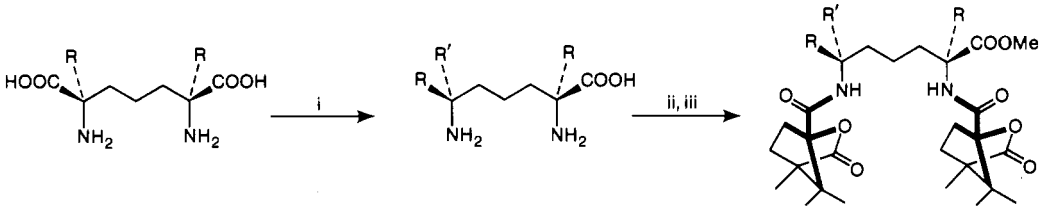
meso-Diaminopimelate decarboxylase was isolated from *Bacillus sphaericus* IFO 3525 by the method described by Asada et al. (1981a) and from wheat germ (*Triticum vulgaris*, Sigma) following the procedure of Mazelis & Creveling (1978). The specific activities of these preparations were 16 units/mg for the bacterial enzyme and 0.017 unit/mg for the wheat germ enzyme. *meso*-DAP decarboxylase activity was estimated by measuring $^{14}CO_2$ evolution from [1,7-^{14}C]diaminopimelate. The assay mixtures (in scintillation vials) contained 50 mM potassium phosphate buffer, pH 6.8, 1.2 mM diaminopimelate, 0.25 μCi of [1,7-^{14}C]diaminopimelate, 50 μM PLP, 1.2 mM EDTA, 3.5 mM DTE, and enzyme in a final volume of 0.86 mL. The caps of the vials contained a 1.5 × 1.5 cm piece of filter paper impregnated with 20 μL of 1 M hyamine hydroxide as a $^{14}CO_2$ trapping agent. The reaction was initiated by the addition of enzyme, and the assay mixture was incubated at 30 °C with continual shaking for 20 min. Reaction was terminated by the addition of 0.2 mL of 10% trichloroacetic acid, and the vials were shaken for an additional 60 min to ensure that $^{14}CO_2$ evolution was complete. The filter paper was removed and counted in 10 mL of ACS scintillation cocktail on a Beckman LS100C scintillation counter. Assays were standardized with an excess of enzyme to completely release and trap CO_2 from substrate. One unit is defined as the amount of enzyme that liberates 1 μmol of CO_2/min. Protein concentrations were estimated with the Bio-Rad protein assay, which is based on the method described by Bradford (1976), using bovine serum albumin as a standard.

General Instrumentation. Infrared spectra were recorded on a Nicolet 7199 FT-IR. Mass spectra were obtained on an A.E.I. MS-50 mass spectrometer. Proton NMR spectra were measured on Bruker WP-80 (80-MHz) and Bruker WH-400 (400-MH) instruments, and ^{13}C NMR spectra were recorded on a Bruker WH-400 (100.6 MHz) instrument. Optical rotation measurements were made on a Perkin-Elmer 141 polarimeter with a 1-cm cell.

Preparation of [2,6-$^2H_2$]Diaminopimelic Acid. The modified procedure of Fujihara & Schowen (1984) was followed. Diaminopimelic acid (a mixture of DD, LL, and meso isomers) (5.0 g, 26 mmol), potassium hydroxide (5.90 g, 105 mmol), and pyridoxal hydrochloride (0.53 g, 2.6 mmol) were dissolved in D_2O (25 mL), and the mixture was stirred at room temperature for 9.5 h. The D_2O was removed by lyophilization, and the residue was dissolved in fresh D_2O (35 mL), heated at reflux for 2 h, cooled, and acidified to pH 5 with concentrated hydrochloric acid. Ethanol (95%) was added until a permanent cloudiness was observed, and cooling and filtration of the mixture yielded 6.25 g of a white solid. This was recrystallized from H_2O/95% EtOH to give 2.38 g (47%) of [2,6-$^2H_2$]diaminopimelic acid, 90% deuterated by ^1H NMR.

Decarboxylation of [2,6-$^1H_2$]Diaminopimelic Acid in D_2O. In a typical experiment, 0.2 unit of wheat germ or 1.4 unit of *B. sphaericus meso*-DAP decarboxylase was dissolved in 25 mL of 10 mM potassium phosphate buffer in D_2O, pD 6.8 (pD is the uncorrected pH meter reading), containing 0.01% 2-mercaptoethanol and 0.1 mM PLP. This solution was concentrated to 2–3 mL in an Amicon ultrafiltration cell equipped with a PM-10 membrane and was then resuspended in 25 mL of fresh buffer. After a second concentration, the enzyme solution was added to 50 mL of 20 mM potassium phosphate buffer, pD 6.8, containing 500 mg of diaminopimelic acid (a mixture of DD, LL, and meso isomers), from which exchangeable protons had been replaced by deuterium through repeated evaporation and dissolution in D_2O. PLP (0.1 mM) and 0.01% 2-mercaptoethanol were added, and the reaction flask was sealed and incubated at 26 °C for 100 h. Lysine and unreacted diaminopimelate were removed from the enzyme by ultrafiltration and applied to an AG 50W-X8 (100–200 mesh) column (H^+ form, 2 × 15 cm, equilibrated with water). The column was washed with water and then with 1 M HCl to separate diaminopimelate from lysine. Fractions containing lysine [detected by TLC on Fixion 50X8 plates developed with 0.07 M sodium citrate buffer (pH 5.1) and sprayed with ninhydrin] were combined and concentrated in vacuo.

Decarboxylation of [2,6-$^2H_2$]Diaminopimelic Acid in H_2O. A typical reaction mixture consisted of 0.2 unit of wheat germ or 1.4 unit of *B. sphaericus meso*-DAP decarboxylase in 50 mL of 20 mM potassium phosphate buffer, pH 7.0, containing 0.01% 2-mercaptoethanol, 0.1 mM PLP, and 500 mg of



1a R=H
1b R=D

2a R=H, R'=D
2b R=D, R'=H

3a R=H, R'=D
3b R=D, R'=H
3c R=R'=H

FIGURE 2: Transformation of DAP and derivatization for NMR analysis. Enzymes and reagents: (i) *meso*-DAP decarboxylase (wheat germ or *B. sphaericus*); (ii) (−)-camphanoyl chloride, NaOH; (iii) diazomethane.

[2,6-$^2H_2$]diaminopimelic acid. After 140 h at room temperature (26 °C), lysine and diaminopimelate were removed by ultrafiltration and separated by ion-exchange chromatography as described above.

Preparation of N,N'-Di-(1S,4S)-camphanoyl-L-lysine Methyl Ester. The procedure followed was adapted from that of Armarego et al. (1976). In a typical experiment, (−)-camphanic acid chloride (134 mg, 0.620 mmol) was dissolved in toluene (0.20 mL), and to this was added L-lysine (labeled or unlabeled) (36 mg, 0.20 mmol), 2 M sodium hydroxide (0.20 mL), and 3 M sodium hydroxide (0.20 mL). The mixture was stirred 2 h at room temperature with intermittent addition of 2 M sodium hydroxide to maintain the pH above 7. The solution was washed with dichloromethane (2 mL) to remove impurities, acidified (pH 1) with 2 M HCl, and extracted with dichloromethane (2 × 5 mL). These extracts were dried (Na_2SO_4) and evaporated to dryness in vacuo. The residue was dissolved in ether (10 mL), and an ethereal solution of diazomethane was added until a persistent yellow color was observed. Concentration of the solution in vacuo left a residue that was purified by flash chromatography (Still et al., 1978) (70% EtOAc/hexane) to yield 82 mg (80%) of *N,N'*-dicamphanoyl-L-lysine methyl ester as a colorless oil, $[\alpha]^{25}_D = -16.8°$ (*c* 1.2, CH_2Cl_2). The unlabeled compound (Figure 2, **3c**) was characterized by IR ($CHCl_3$ cast, 3370, 1790, 1740, 1670, and 1530 cm^{-1}), mass spectrometry (exact mass 520.2789; calculated for $C_{27}H_{40}N_2O_8$ 520.2785), microanalysis (Calcd for $C_{27}H_{40}N_2O_8$: C, 62.29; H, 7.74; N, 5.38. Found: C, 62.63; H, 7.73; N, 5.05), ^1H NMR, and ^{13}C NMR. The deuterium-labeled compounds were prepared analogously and possessed the expected spectral and chromatographic properties.

NMR Spectra. ^1H, ^{13}C, and ^1H–^{13}C shift correlation spectra were obtained on a Bruker WH-400 instrument. Typically, 20–50 mg of labeled material was diluted with 10–20 mg of unlabeled material in 0.5 mL of C_6D_6 containing Me_4Si and 15 drops of C_6F_6 in 5-mm tubes. Two-dimensional ^1H–^{13}C shift correlation experiments with ^2H decoupling (^{19}F lock on C_6F_6) used literature (Levitt et al., 1983; Pegg & Bendall, 1983; Nakashima et al., 1984a,b) pulse sequences with the following parameters: acquisition time 0.098 s, f_1 (^1H) 1724 Hz, f_2 (^{13}C) 5208 Hz, relaxation delay 3 s between scans, 64–290 scans per t_1 increment, 1K FIDs for 64 values of t_1, zero filling to 512 points in f_1 and 2K in f_2, and line broadening 6.0 and 5.0 for f_1 and f_2, respectively.

RESULTS

Since the signals due to the terminal C-6 methylene hydrogens of lysine are not resolved in ^1H NMR, spectroscopic analysis of the stereochemistry of deuterium labeling at that site requires derivatization. The bis-(−)-camphanamide was chosen since extensive precedent (Parker, 1983) shows that

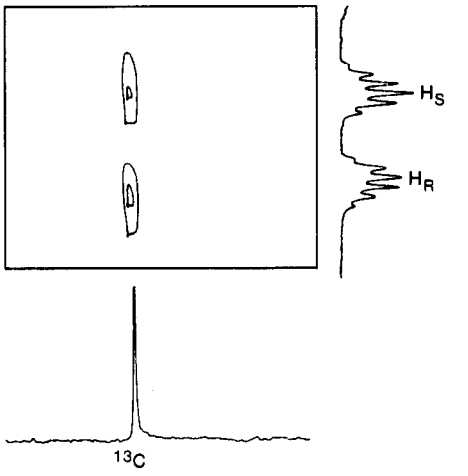


FIGURE 3: Expansion of the ^1H–^{13}C NMR shift correlation spectrum of unlabeled lysine derivative **3c** showing the C-6 methylene region. The corresponding ^1H NMR spectrum is shown along the vertical hydrogen chemical shift axis, and the ^1H-decoupled ^{13}C NMR spectrum of C-6 is displayed along the horizontal carbon chemical shift axis (δ increases right to left).

diastereotopic hydrogens on a methylene group adjacent to nitrogen in such compounds give well-resolved NMR signals in deuterated benzene. Furthermore, in all cases examined so far, the *pro-S* hydrogen resonance always appears downfield from that of the *pro-R*. The free acid of the bis-camphanamide proved unexpectedly unstable and difficult to purify, so the methyl ester (Figure 2, **3a–c**), which can be purified by flash chromatography (Still et al., 1978), was prepared by treatment of the free acid with diazomethane. The diastereotopic terminal methylene hydrogen resonances of this compound are well resolved in the 400-MHz NMR in deuteriobenzene solution and appear at δ 3.26 and 3.11. Application of a normal ^1H–^{13}C heteronuclear NMR shift correlation experiment [Kessler et al. (1983) and references cited therein] to unlabeled compound **3c** showed that the C-6 hydrogens are directly attached to the carbon whose ^{13}C NMR resonance is at δ 38.8. Figure 3 shows a small portion (C-6 methylene region) of the correlation map.

In order to confirm the unusual inversion of stereochemistry catalyzed by *B. sphaericus meso*-DAP decarboxylase reported earlier (Asada et al., 1981), we repeated the isolation of this enzyme and treated it with unlabeled diaminopimelate (mixture of isomers) in D_2O. Only the R_D center of the *meso* isomer is decarboxylated. The lysine obtained from this reaction is expected to contain deuterium in the *pro-R* position and a proton in the *pro-S* position (compound **2a**, Figure 2) and to provide the bis-camphanamide methyl ester **3a** upon derivatization. The deuterium-decoupled ^1H–^{13}C NMR shift correlation spectrum of a mixture of this derivative and the corresponding unlabeled material **3c** (added as internal

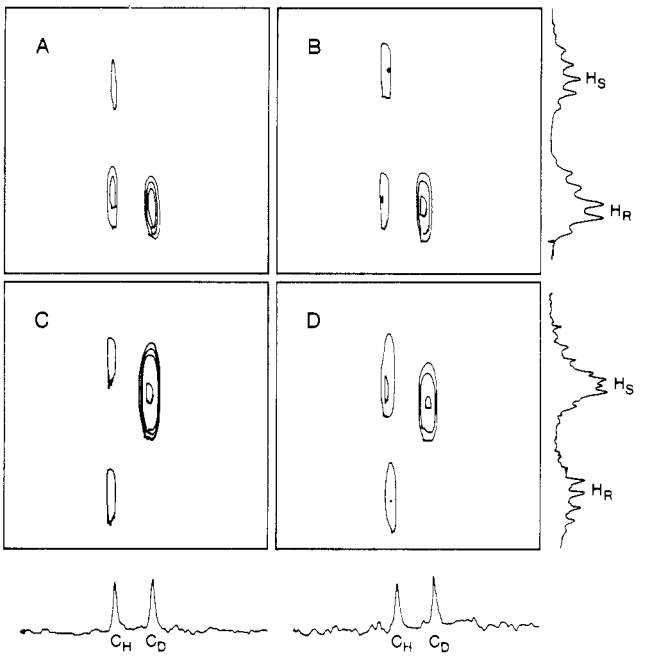


FIGURE 4: Corresponding portions (see Figure 3) of the deuterium-decoupled ^1H–^{13}C NMR shift correlation maps of deuterated lysine derivatives **3a** and **3b** diluted with unlabeled standard **3c**. (A) From wheat germ *meso*-DAP decarboxylase and [2,6-$^2H_2$]-DAP in H_2O. (B) From *B. sphaericus meso*-DAP decarboxylase and [2,6-$^2H_2$]-DAP in H_2O. (C) From wheat germ *meso*-DAP decarboxylase and DAP in D_2O. (D) From *B. sphaericus meso*-DAP decarboxylase and DAP in D_2O.

standard) is shown in Figure 4D. The downfield ^{13}C signal (C_H) arises from unlabeled material and correlates to both diastereotopic protons (cf. Figure 3), while the upfield ^{13}C resonance (C_D) comes from deuterated material and is shifted (0.30 ppm) due to the α-deuterium isotope effect (Maciel et al., 1967; Garson & Staunton, 1979). This upfield carbon resonance correlates only to the downfield diastereotopic hydrogen peak, which corresponds to the *pro S* hydrogen at C-6 (Parker, 1983). Hence, within experimental error, all labeled molecules have deuterium in the *pro R* position at C-6 in complete accord with earlier results of Soda and co-workers (Asada et al., 1981). In the complementary experiment, L-lysine obtained from reaction of [2,6-$^2H_2$]diaminopimelate with *B. sphaericus meso*-DAP decarboxylase in H_2O was shown to have the opposite stereochemistry (compound **2b**). The corresponding portion of the deuterium-decoupled correlation spectrum of its derivative **3b** (with unlabeled internal standard) is shown in Figure 4B. Here, the signal arising from the deuterated carbon (C_D) correlates only to the most upfield proton peak, and thus, this carbon must bear a hydrogen in the *pro-R* position and a deuterium in the *pro-S* position. The results clearly confirm inversion of configuration in the *B. sphaericus meso*-DAP decarboxylase reaction.

The corresponding enzyme from wheat germ (*T. vulgaris*) was isolated according to literature procedures (Mazelis & Creveling, 1978). Separate incubations of this eukaryotic DAP decarboxylase with unlabeled diaminopimelate in D_2O and with [2,6-$^2H_2$]diaminopimelate in H_2O gave L-lysine samples that were derivatized and analyzed as described above. Comparison of the corresponding NMR correlation spectra (Figure 4C,A) to the *B. sphaericus* results (Figure 4D,B) demonstrates that the wheat germ enzyme operates with the same unusual stereochemistry, net inversion of configuration.

It is interesting to note that the CHD signal in the correlation spectra of the deuterated compounds is also shifted slightly upfield on the hydrogen chemical shift axis with respect to the corresponding signal in the unlabeled compound. This may be at least partly due to a deuterium-induced isotope shift on the ^1H NMR resonance position (Bernheim & Batiz-Hernandez, 1966). This effect is not readily noticeable in the ^1H NMR spectrum along the vertical axis of the plot, due to the width of the proton signals and the small magnitudes of such isotope shifts.

Discussion

In PLP-dependent enzyme catalyzed reactions, the bond to be broken in the substrate is expected to be perpendicular to the plane of the conjugated π system of the substrate–PLP complex (Dunathan, 1966; Floss & Vederas, 1982; Palcic & Floss, 1985). In decarboxylases, it is the bond between C_α and the carboxyl carbon that must be aligned in this way (Figure 5). Where retention of configuration occurs, protonation must take place on the same face of the planar carbanionic intermediate as did decarboxylation; this implies that one face only of the PLP–substrate complex is available to solvent (Dunathan & Voet, 1974). However, in the case of either *B. sphaericus* or wheat germ *meso*-DAP decarboxylase, the proton must attach to the opposite face of the carbanionic intermediate to that from which the carboxyl group left (Figure 5). This may be explained by one of two hypotheses proposed by Asada et al. (1981b). The incoming proton may be donated from a catalytic group on the enzyme positioned on the opposite face of the PLP–substrate complex to that from which the carboxyl group leaves. This group would then be reprotonated by solvent later in the catalytic cycle. Alternatively, the PLP–substrate complex may undergo a large conformational change after decarboxylation to expose its other face to solvent protons, much like the "swinging-door" mechanism proposed for racemases [Henderson & Johnston, 1976; cf. Shen et al. (1983)]. In either case, Figure 5 depicts only one of two possible initial conformations for reaction because the diastereomeric orientation resulting from 180° rotation about the C_α–N bond would also have the C_α carboxyl bond perpendicular to the plane of the π system. Beginning with such a conformation, the decarboxylation would occur above the plane and protonation would proceed from below.

Presently it is not proven that both DAP decarboxylases possess identical substrate–cofactor complex orientations or that they employ precisely the same type of protonation to achieve inversion. However, this unusual stereochemical outcome in combination with the high specificity for only the meso isomer of DAP and similar behavior with potential inhibitors (unpublished results) strongly support identical mechanisms for both enzymes. It is tempting to hypothesize that both the eukaryotic (wheat germ) and prokaryotic (*B. sphaericus*) *meso*-DAP decarboxylases are structurally similar

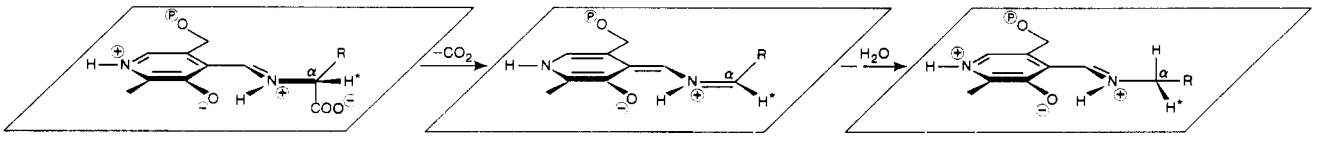


FIGURE 5: Stereochemical orientation of the substrate–cofactor complex bound to *meso*-DAP decarboxylase during reaction. A diastereomeric orientation in which the C_α–N bond is rotated 180° is also possible (vide infra).

and evolved in a divergent fashion from a "grandfather" enzyme (Dunathan & Voet, 1974). If convergent evolution had occurred, it seems more likely that the stereochemical course of the wheat germ enzyme would be retention like that of other PLP-dependent decarboxylases. It will be interesting to see whether other *meso*-DAP decarboxylases operate with the same inversion of stereochemistry and whether all possess homologous amino acid sequences (Stragier et al., 1983). The NMR methodology introduced in the present work should prove useful not only for studies on decarboxylases but more generally for determination of stereospecific deuterium labeling at diastereotopic methylene hydrogens.

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Registry No. **1a**, 922-54-3; **1b**, 96328-50-6; **2a**, 96392-62-0; **2b**, 96392-99-3; **3a**, 96328-51-7; **3b**, 96328-52-8; **3c**, 96328-53-9; (−)-camphanoyl chloride, 39637-74-6; L-lysine, 56-87-1; diaminopimelate decarboxylase, 9024-75-3.

REFERENCES

Akhtar, M., Emery, V. C., & Robinson, J. A. (1984) *New Compr. Biochem. 6*, 303–372.

Armarego, W. L. F., Milloy, B. A., & Pendergast, W. (1976) *J. Chem. Soc., Perkin Trans. 1*, 2229–2237.

Asada, Y., Tanizawa, K., Kawabata, Y., Misono, H., & Soda, K. (1981a) *Agric. Biol. Chem. 45*, 1513–1514.

Asada, Y., Tanizawa, K., Sawada, S., Suzuki, T., Misono, H., & Soda, K. (1981b) *Biochemistry 20*, 6881–6886.

Asada, Y., Tanizawa, K., Nakamura, K., Moriguchi, M., & Soda, K. (1984) *J. Biochem. (Tokyo) 95*, 277–282.

Bernheim, R. A., & Batiz-Hernandez, H. (1966) *J. Chem. Phys. 45*, 2261–2269.

Bradford, M. M. (1976) *Anal. Biochem. 72*, 248–254.

Dunathan, H. C. (1966) *Proc. Natl. Acad. Sci. U.S.A. 55*, 712–716.

Dunathan, H. C. (1971) *Adv. Enzymol. Relat. Areas Mol. Biol. 35*, 79–134.

Dunanthan, H. C., & Voet, J. G. (1974) *Proc. Natl. Acad. Sci. U.S.A. 71*, 3888–3891.

Floss, H. G., & Vederas, J. C. (1982) in *Stereochemistry* (Tamm, C., Ed.) pp 161–199, Elsevier Biomedical Press, Amsterdam.

Fujihara, H., & Schowen, R. L. (1984) *J. Org. Chem. 49*, 2819–2820.

Garson, M. J., & Staunton, J. (1979) *Chem. Soc. Rev. 8*, 539–561.

Henderson, L. L., & Johnston, R. B. (1976) *Biochem. Biophys. Res. Commun. 68*, 793–798.

Kato, Y. (1979) *Mem. Tokyo Univ. Agric. 21*, 57–96.

Kessler, H., Bernd, M., Kogler, H., Zarbock, J., Sorensen, O. W., Bodenhausen, G., & Ernst, R. R. (1983) *J. Am. Chem. Soc. 105*, 6944–6952.

Lakshman, M., Shenoy, B. C., & Rao, M. R. R. (1981) *J. Biosci. 3*, 89–103.

Levitt, M. M., Sorensen, O. W., & Ernst, R. R. (1983) *Chem. Phys. Lett. 94*, 540–542.

Liu, H. W., Auchus, R., & Walsh, C. T. (1984) *J. Am. Chem. Soc. 106*, 5335–5348.

Maciel, G. E., Ellis, P. D., & Hofer, D. C. (1967) *J. Chem. Phys. 71*, 2160–2164.

Mazelis, M., & Creveling, R. K. (1978) *J. Food Biochem. 2*, 29–37.

Nakashima, T. T., John, B. K., & McClung, R. E. D. (1984a) *J. Magn. Reson. 57*, 149–151.

Nakashima, T. T., John, B. K., & McClung, R. E. D. (1984b) *J. Magn. Reson. 59*, 124–131.

Nakazawa, H., Ajisaka, K., Kainosho, M., Sawada, S., & Yamada, H. (1981) *Agric. Biol. Chem. 45*, 2553–2557.

Orr, G. R., & Gould, S. J. (1982) *Tetrahedron Lett.*, 3139–3142.

Orr, G. R., Gould, S. J., Pegg, A. E., Seely, J. E., & Coward, J. K. (1984) *Bioorg. Chem. 12*, 252–258.

Palcic, M. M., & Floss, H. G. (1985) in *Pyridoxal Phosphate and Derivatives* (Dolphin, D., Poulson, R., & Avramovic, O., Eds.) Wiley, New York (in press).

Parker, D. (1983) *J. Chem. Soc., Perkin Trans. 2*, 83–88.

Patte, J. C. (1983) *Biotechnol. Ser. 3*, 213–228.

Pegg, D. T., & Bendall, M. R. (1983) *J. Magn. Reson. 55*, 114–127.

Richards, J. C., & Spenser, I. D. (1982) *Can. J. Chem. 60*, 2810–2820.

Robins, D. J. (1983) *Phytochemistry 22*, 1133–1135.

Rosenthal, G. A. (1982) *Plant Nonprotein Amino and Imino Acids, Biological, Biochemical and Toxicological Properties*, pp 168–171, Academic Press, New York.

Rosner, A. (1975) *J. Bacteriol. 121*, 20–28.

Santaniello, E., Kienle, M. G., Manzocchi, A., & Basisio, E. (1979) *J. Chem. Soc., Perkin Trans. 1*, 1677–1679.

Shen, S. J., Floss, H. G., Kumagai, H., Yamada, H., Esaki, N., Soda, K., Wasserman, S. A., & Walsh, C. (1983) *J. Chem. Soc., Chem. Commun.*, 82–83.

Snell, E. E. (1982) *Adv. Exp. Med. Biol. 148*, 257–272.

Sodek, L. (1978) *Rev. Bras. Bot. 1*, 65–69; *Chem. Abstr. 90*, 50398*q*.

Still, W. C., Kahn, M., & Mitra, A. (1978) *J. Org. Chem. 43*, 2923–2925.

Stragier, P., Danos, O., & Patte, J. C. (1983) *J. Mol. Biol. 168*, 321–331.

Trimble, L. A., Reese, P. B., & Vederas, J. C. (1985) *J. Am. Chem. Soc. 107*, 2175–2177.

Vogel, H. J., & Hirvonen, A. P. (1971) *Methods Enzymol. 17*, 146–150.

White, P. J. (1971) *Methods Enzymol. 17*, 140–145.

White, P. J. (1983) *J. Gen. Microbiol. 129*, 739–749.

Wigle, I. D., Mestichelli, L. J. J., & Spenser, I. D. (1982) *J. Chem. Soc., Chem. Commun.*, 662–664.