Stereochemistry of Decarboxylation Reactions Catalyzed by a Constitutive Aromatic L-Amino Acid Decarboxylase

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The stereochemistry of the decarboxylation reaction catalyzed by an aromatic L-amino acid decarboxylase, purified from Micrococcus perectreus, was studied using stereospecifically deuterium labelled phenylalanine (Phe). The $^1$H NMR spectrum of $[1,2-2H_2]$-l-phenethylamine enzymatically derived from $(2S,3R)-[3-2H]$-Phe in $^2$H$_2$O was compared with that of $[1-2H]$-l-phenethylamine from unlabelled Phe in $^2$H$_2$O. The results clearly indicate that the decarboxylation reaction of this enzyme proceeds exclusively through a course in which the configuration at C-2 of Phe is retained.

In the previous paper$^{1}$ we have shown that a constitutive aromatic L-amino acid decarboxylase from Micrococcus perectreus is a pyridoxal phosphate-dependent enzyme which catalyzes the following two reactions: (1) $\alpha$-decarboxylation reaction of various L-amino acids including Phe, Tyr, DOPA, Trp, and 2-methyl derivatives of these amino acids (reaction type 1 and 2$^{2,3}$; (2) trans-aminase dependent decarboxylation of various 2-methyl aromatic L-amino acids (reaction type 3).$^4$

$\text{Ar-CH}_2-\text{CH}-\text{CO}_2^- + \text{NH}_3 \rightarrow \text{Ar-CH}_2-\text{CH}_2 + \text{CO}_2 + \text{NH}_3$ (1)

$\text{Ar-CH}_2-\text{CH}-\text{CO}_2^- + \text{PLP} \rightarrow \text{Ar-CH}_2-\text{CH}_2 + \text{PMP} + \text{CO}_2$ (2)

All of these reactions can be explained by a widely recognized mechanism shown in Fig. 1.$^4-6$ As shown in this scheme, the azomethin bond between apoenzyme and PLP is being replaced in the ternary complex I by the bond between various substrates and PLP. After the decarboxylation takes place in the substrates portion of I, the intermediate II can be hydrolyzed, in principle, via two tautomeric forms, III and IV. These two pathways should lead to the different products as shown in Fig. 1. Either of which, namely pathway a and pathway b, dominates depending primarily on the reaction conditions and also on the nature of substrates. Types 1 and 2 re-
actions are considered to occur through the intermediate III, which can regenerate the active L-amino acid decarboxylase after being hydrolyzed. Type 3 reaction, which could only be seen for aromatic 2-methyl-L-amino acids as substrates, must include the intermediate IV. This intermediate, however, does not regenerate PLP, and PMP is formed stoichiometrically. It seems to be an important but unexplored issue on the reaction mechanism in which the configuration of C-2 in the substrate amino acids retains or inverts (or racemizes) during the enzymatic reactions. In this paper, we describe a stereochemical study by $^1$H NMR on the aromatic L-amino acid decarboxylase catalyzed reaction of L-(2S)-Phe and dl-{(2S,3R); (2R,3S)}-[3-$^2$H]-Phe to yield [1-$^2$H]-β-phenethylamine and [1,2-$^2$H$_2$]-β-phenethylamine, respectively.

**EXPERIMENTAL PROCEDURE**

**Materials.** L-(2S)-Phe was obtained from Ajinomoto Co. Inc., and dl-{(2S,3R); (2R,3S)}-[3-$^2$H]-Phe was synthesized according to the method of Kirby et al. The deuterium labelled Phe has been shown to be highly stereospecific and no attempt was made to resolve into L-isomer because the enzymatic reactions do not take place and are not inhibited by D-isomer. PLP was purchased from Dai-nippon Pharmaceutical Co. Ltd., Osaka. NaOD and $^2$H$_2$O were purchased from Merck and were more than 99.5 atomic %.

**Spectral determination.** $^1$H NMR spectra were determined on a Varian XL-100-15 spectrometer at 100 MHz in the CW mode. Deuterium decoupling was used, when necessary, to simplify the spectra. Mass spectra were measured using a Shimadzu LKB-9000S.

**Preparation of aromatic L-amino acid decarboxylase.** Aromatic L-amino acid decarboxylase which was used for the formation of β-phenethylamine from L-Phe was purified over 95% from the cell extract of Micrococcus percireus (AJ 1065) according to the method described in the previous paper. The enzyme lyophile was prepared as follows: One ml of the aqueous enzyme solution contain

**Fig. 1.** Schematic Representation of the Plausible Reaction Pathways of Decarboxylation Reactions for Various Aromatic Amino Acids and Their 2-Methyl Derivatives Catalyzed by Aromatic L-Amino Acid Decarboxylase. Noncovalent bindings between the enzyme and substrates are not shown in this figure.
ing 40 units of aromatic L-amino acid decarboxylase was added to 100 mg monosodium glutamate and 1 mg PLP. After being lyophilized, the residue was dissolved in 3 ml $^2$H$_2$O and then lyophilized again. This procedure was repeated twice in order to replace water and exchangeable protons with $^3$H$_2$O and deuterons.

Enzymatic decarboxylation of L-Phe and DL-((2S,3R); (2R,3S))-[3-$^2$H]-Phe. The reaction was carried out at 30°C for 20 hr in a 10 ml $^2$H$_2$O solution containing either 50 mg of L-Phe or 100 mg of DL-((2S,3R); (2R,3S))-[3-$^2$H]-Phe (50 mg of L-isomer), 1 mg PLP, 100 mg K$_2$HPO$_4$, 20 units of the “deuterated” enzyme lyophile described above. The pH (uncorrected pH meter reading) was adjusted to 8.5 with 2 N-NaOD. The reaction was terminated by heating the solution up to 80°C for 20 min and the denatured enzyme was removed by filtration. The filtrate was acidified (pH ≈ 1) with 2 N HCl and extracted twice with 10 ml ether. The aqueous layer was made alkaline (pH ≈ 13.5) with 6 N-NaOH and the amine was extracted twice with a 20 ml ether. After being dried in vacuo, the ethereal extract gave about 40 mg of β-phenethylamine for both experiments.

RESULTS AND DISCUSSIONS

β-Phenethylamines obtained by the enzymatic decarboxylation of Phe and [3-$^2$H]-Phe in $^2$H$_2$O gave single spot on TLC, of which $R_f$ value was identical to that of the authentic sample. In order to determine the deuterium contents of these amines by mass spectrometry, they were acetylated in pyridine-acetic acid.

![Fig. 2](image_url)

**Fig. 2.** Deuterium Decoupled 100 MHz Proton Nuclear Magnetic Resonance Spectra of [A] (1R)-[1-$^2$H]-β-phenethylamine·HCl and [B] (1R, 2R)-[1,2-$^2$H$_2$]-β-phenethylamine·HCl. Measured in $^2$H$_2$O at room temperature. Chemical shift is shown from the internal sodium trimethylsilylpropionate-$^2$H$_4$ (TMSP). The inserted spectra are the expanded portions of proton resonances attached to C-1 ($H_1$). This part of the spectrum [A] is due to the A nucleus of AB$_2$ spin systems, and the spacing of the outermost peaks, 14.6 Hz, should therefore be the sum of $J_{H_1,H_2}$ and $J_{H_1,H_2}$. In the case of spectrum [B], however, it is an B part of AB spin system, and the spacing of doublet, 8.3 Hz, should be $J_{H_1,H_2}$. 

anhydride. Each of the N-acetyl-β-phenethylamines gave very similar fragmentation patterns and the molecular peaks appeared at m/z 163, 164, and 165 for the N-acetyl derivatives for authentic sample, the amines from Phe in 2H2O and from [3-2H]-Phe in 2H2O, respectively. It is therefore clear that β-phenethylamine from Phe contains one deuteron and that the amine from [3-2H]-Phe contains two deuterons. Both amines were identified to be [1-2H]-β-phenethylamine and [1,2-2H2]-β-phenethylamine from their spectral data.

1H NMR spectra of [1-2H]-β-phenethylamine and [1,2-2H2]-β-phenethylamine are shown in Fig. 2. Note that 1H-2H spin couplings were decoupled by deuterium irradiation. Two vicinal 1H-1H coupling constants, 6.3 and 8.3 Hz, were observed for [1-2H]-β-phenethylamine, while only one coupling constant, 8.3 Hz, was observed for [1,2-2H2]-β-phenethylamine. The result immediately excludes the possibility of racemization at C-1 configuration during decarboxylation, since if so there should be observed two coupling constants also for [1,2-2H2]-β-phenethylamine. Obviously it was not the case and the stereochemistry of decarboxylation therefore is either complete retension or inversion at the C-2 configuration in Phe. As these two possible pathways lead to different configurations at C-1 in the amine, the two deuterated amines must be diastereomeric to each other. Thus we could in principle differentiate these two possibilities by 1H NMR spectroscopy. We will discuss this later on. To avoid some possible confusion, we ought to mention that the carbon atom carrying the amino group is denoted C-2 in Phe and C-1 in β-phenethylamine in the following discussion.

When L-(2S)-Phe is being decarboxylated in 2H2O, (1R)-[1-2H]-β-phenethylamine or (1S)-[1-2H]-β-phenethylamine should be obtained depending on the stereochemistry is retension or inversion, respectively. Note that the configurational notation of C-1 in the amine is different from C-2 in Phe, because of the priority of deuteron is less than that of carboxylate. Needless to say, the 1H NMR of these two enantiomers must be exactly identical in usual conditions. Situation, however, is entirely different when L-(2S,3R)-[3-2H]-Phe is being decarboxylated in 2H2O. Here we ignore D-isomer actually exists in the deuterated Phe, since it does not do anything in the enzymatic reaction (we are concerning with). As the configuration of C-3 in Phe retains during the reaction, C-2 in β-phenethylamine must be R. So the configuration of two possible [1,2-2H2]-β-phenethylamine should be either (1R,2R) (retension) or (1S,2R) (inversion). The problem is how to differentiate these two isomers by 1H NMR spectroscopy without having authentic reference amine, which is obviously difficult to prepare.

In solutions, β-phenethylamine exists in the mixture of three staggered structures, which interconvert rapidly. The Newman pro-
jections of these three rotamers are given in Fig. 3. Here the suffix r and s denote pro-R and pro-S protons respectively. In (1R,2R)- and (1S,2R)-[1,2-2H]-β-phenethylamine, H1r, H2r, and H1s, H2s, are replaced with deuterons, respectively. As the observed vicinal 1H-1H spin coupling constants can be expressed by the weighted average of gauche and trans vicinal coupling constants, Jg and Jt, the expected coupling constants, Jret (the stereochemistry of decarboxylation is retention) and Jinv (inversion) can be represented by the following equations.

\[
J_{\text{ret}} = p_1 J_g + p_\Pi J_g + p_\Pi J_t
\]
\[
J_{\text{inv}} = p_1 J_t + p_\Pi J_g + p_\Pi J_g
\]
\[
p_1 + p_\Pi + p_\Pi = 1; \quad p_1 = p_\Pi,
\]
where p denotes the fractional population of each rotamer in Fig. 3.

The relative magnitude of the expected vicinal coupling constants for retention and inversion stereochemistry can be predicted by considering the sign of \(J_{\text{ret}} - J_{\text{inv}}\), which is given below. Since two bulky substituents tend to be in trans position, \(p_\Pi\) should be very probably greater, at least to some extent, than \(p_1\) or \(p_\Pi\). Keeping this assumption in mind, we could predict \(J_{\text{ret}} > J_{\text{inv}}\), since \(J_g < J_t\).

The experimental value of 8.3 Hz was greater than the other possible value of 6.3 Hz (vide supra). Therefore, the configuration of [1,2-2H]-β-phenethylamine obtained by the enzymatic decarboxylation of (2S,3R)-[3-2H]-Phe in 2H2O must be (1R,2R). We thus concluded the stereochemistry of the reaction to be retention.

Finally, since the enzymatic reaction proceeds quite facilely and stereospecifically, it would provide a versatile way to prepare various (1R)-[1-2H]- or (1R)-[1-3H]-β-aryl amines, which may be useful in various biochemical studies.

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