Stereochemistry of Decarboxylation of Arylmalonate Catalyzed by Mutant Enzymes

Kenji Miyamoto, Tomomi Tsutsumi, Yosuke Terao, and Hiromichi Ohta*
Department of Biosciences and Informatics, Keio University,
3-14-1 Hiyoshi, Kohoku-ku, Yokohama 223-8522

(Received February 16, 2007; CL-070184; E-mail: hohta@bio.keio.ac.jp)

The enantiotopos differentiating selectivity of the mutant arylmalonate decarboxylase (S36N/G74C/C188S), which catalyzes asymmetric decarboxylation of arylmethylmalonates to give the corresponding arylpropionates, was revealed to be the same as that of the wild type enzyme, in spite of the fact that two enzymes gave the opposite enantiomer with each other.

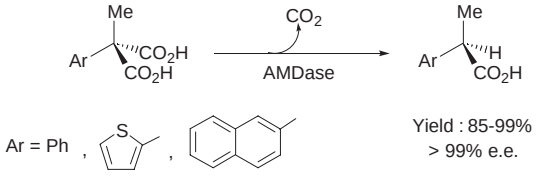
Arylmalonate decarboxylase (AMDase, EC. 4.1.1.76) is an enzyme which catalyzes asymmetric decarboxylation of α-aryl-α-methylmalonate into (*R*)-α-arylpropionate (Scheme 1, configuration is *S* when Ar = thienyl, because of the priolity rule).[1]

It has been already clarified that the pro-*R* carboxyl group is removed as carbon dioxide, and hence it can be said that the reaction proceeds with inversion of configuration.[2]

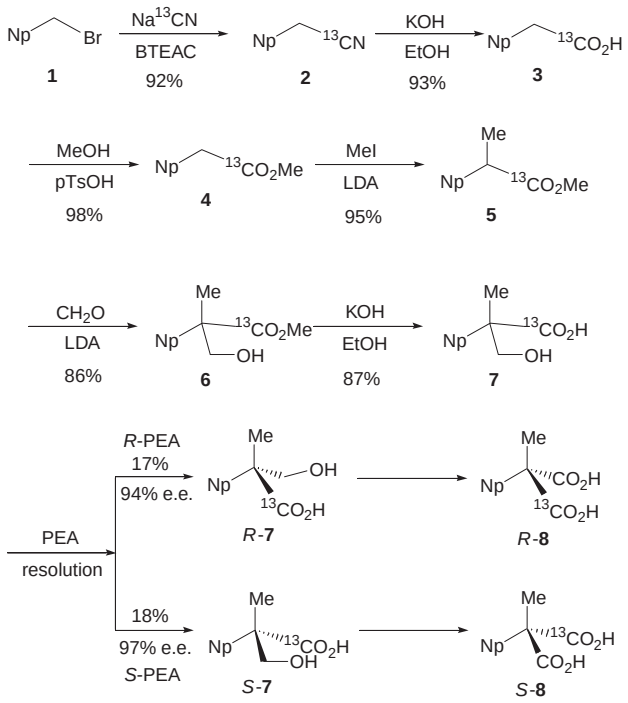
Recently, we have succeeded in preparation of mutant enzymes of which enantioselectivities are opposite to that of wild type enzyme, via site-directed mutagenesis based on the comparison of the reaction mechanism of this enzyme with that of glutamate racemase.[3] The introduction of single mutation (G74C) resulted in the formation of racemic products.[4] The introduction of double mutations (G74C/C188S) resulted in the inversion of enantioselectivity to give (*S*)-α-arylpropionate.[4] In addition, the introduction of another mutation (S36N) to the double mutant enhanced the activity of the mutant enzyme.[5]

It is an interesting problem how the mutant enzymes (G74C, S36N/G74C/C188S) differentiate the prochirality of the substrates. Thus, both enantiomers of methyl-β-naphthylmalonate containing ^{13}C on either one of the two carboxyl groups were synthesized and subjected to the reaction with mutant enzymes.

The chiral malonate was prepared as follows. As shown in Scheme 2, ^{13}C-containing cyano group was introduced to 2-(bromomethyl)naphthalene (**1**) via S_N2 reaction with $Na^{13}CN$.[6] The resulting nitrile **2** was converted to 2-hydroxymethyl-2-(2-naphthyl)propionate (**7**) by the sequence of conversion of the cyano group to a carboxyl group, esterification, methylation of the α-position, and hydroxymethylation with gaseous formaldehyde.[7] The optical resolution of [1-^{13}C] 2-hydroxymethyl-2-(2-naphthyl)propionate was carried out by the formation of salts between *R*- and *S*-phenylethylamine.[8] The diastereomeric salt was recrystallized from acetone. The absolute configuration of one diasteromer containing *R*-amine was determined by X-ray crystallographyic analysis.[9] Jones' oxidation of *R*- and *S*-**7** gave *R*- and *S*-**8**, respectively.



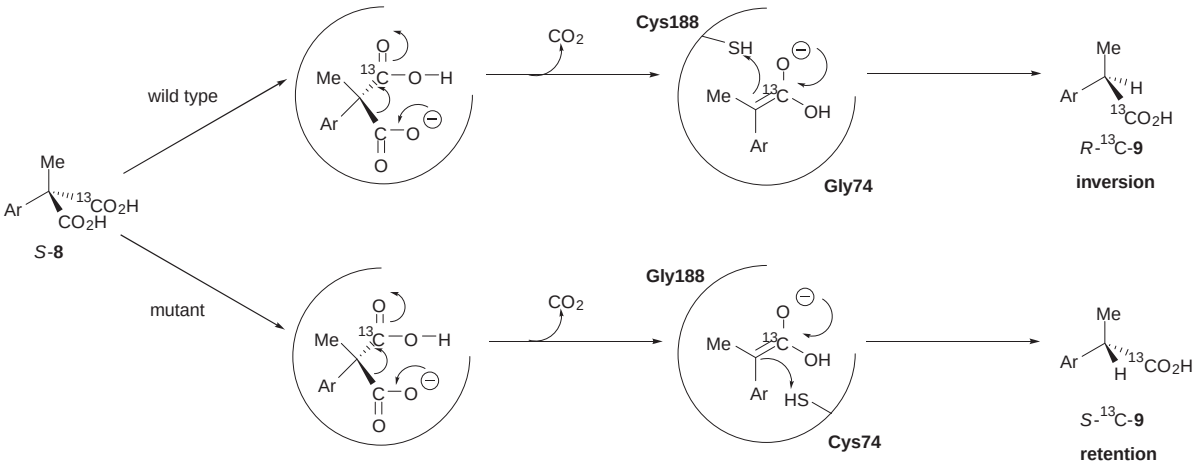
Scheme 1. Asymmetric decarboxylation of AMDase.



Scheme 2. Preparation of the substrate.

The enzymatic reaction was performed at 37 °C for 12 h in 1 mL of 250 mM Tris-HCl buffer (pH 8.5) containing 50 mM of KOH, 10 mg of the mutant enzyme (ca. 50 unit), and [1-^{13}C]-**8** (10 mg, final conc. 50 mM). As the activity of the triple mutant enzyme is quite low compared to that of wild type enzyme[5] (the ratio of *k*cat/*K*m is 1.43:100), a large amount of the enzyme was used for the smooth reaction. The product was isolated as the corresponding methyl ester.[10] When *S*-**8** was employed as the substrate for the reaction with G74C and S36N/G74C/C188S mutant enzymes, ^{13}C was completely retained in the decarboxylated product (^{13}C-**9**). The presence of ^{13}C was confirmed by ^{13}C NMR, ^{1}H NMR, and MS spectroscopy. On the contrary, reaction of *R*-**8** resulted in the formation of **9** containing ^{13}C only within the amount of natural abundance (Figure 1).

The above observation leads us to conclude that the pro-*R* carboxyl group was removed as carbon dioxide by G74C and S36N/G74C/C188S mutants. This is the same enantiotopos selectivity as that of the wild type enzyme, regardless to the fact that the resulting monocarboxylic acids are racemic or exhibit the opposite configuration. Thus, it can be said that the reactions by the mutant enzyme proceeds with retention of configuration.



Scheme 3. The reaction mechanism of asymmetric decarboxylation.

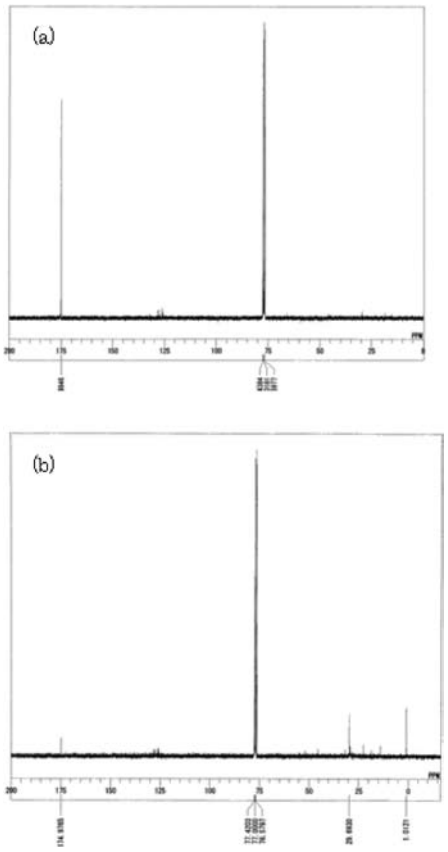


Figure 1. Chart of ^{13}C NMR (a) product by S36N/G74C/C188S mutant enzyme and (*S*)-**8**. (b) product by S36N/G74C/C188S mutant enzyme and (*R*)-**8**.

The reactions in total suggest the presence of an amino acid residue that selectively activates the pro-*R* carboxyl group of the substrate. The X-ray crystallography of wild type enzyme is now under way. If the tert-structure of the enzyme is resolved, it will be possible to discuss the reaction mechanism in more detail.

The reaction mechanism can be estimated as shown in Scheme 3.[11] The first step of the reaction is believed to be the activation of the pro-*R* carboxyl group, which is decarboxylated. The second step will be enantioface-differentiating protonation from Cys188 (wild type) and Cys74 (mutant), respectively, to give the observed product. The stereochemical course of the reactions of wild type and mutant enzymes can be summarized as follows. The enantiotopos differentiation of the first step is the same and the enantioface differentiation of the second step is opposite. As the enantioselectivity of the second step determines the configuration of the final product, wild type and mutant enzyme give the opposite enantiomer. For this type of enantioselective mechanism to work actually, there should be an amino acid residue(s) which selectively catalyzes the decarboxylation of pro-*R* carboxyl group.

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Published on the web (Advance View) April 14, 2007; doi:10.1246/cl.2007.656