The Aldol Type Reaction Catalyzed by Arylmalonate Decarboxylase —A Decarboxylase can Catalyze an Entirely Different Reaction, Aldol Reaction—

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The catalytic promiscuity of AMDase was demonstrated using a well-designed substrate based on the consideration of the reaction mechanism. As the reaction catalyzed by AMDase is supposed to proceed via an enolate intermediate, we expected that the enzyme promotes the aldol type reaction when an acceptor is properly arranged, which turned out to be true.

Enzymes are becoming more efficient and useful catalysts not only in biochemistry or agricultural chemistry but also in organic synthetic chemistry. It had been believed that they could transform only natural substrates in aqueous solutions and catalyze only one reaction. However, it has been revealed that substrate specificities of enzymes are broad and many enzymes can catalyze the transformation of unnatural substrates. Also, some enzymes, for example lipases and esterases, were turned out to be able to catalyze the reactions in organic solvents. These properties of enzymes are called as enzymatic promiscuity.[1] It is said that the broad substrate specificities of enzymes is called as the first promiscuity and the flexibility of reaction conditions of enzymes is called as the second promiscuity. Recently, the third enzymatic promiscuity has been proposed, i.e., the flexibility of the reactions catalyzed by enzymes. For example, it was reported that a lipase was endowed a new catalytic activity, i.e., aldolase activity by changing an amino acid residue in the active site.[2] In this case, the mutation was introduced into the critical amino acid residue to change the function of the lipase by considering the reaction mechanism. Also, there are some reports recently showing the enzymes' promiscuities that a single active site of one enzyme can catalyze more than one distinct chemical transformations.[3] Over the last few years, evidences have mounted that such catalytic promiscuity exists not just among a few enzymes but is rather common.[4]

In this letter, we would like to describe the promiscuity of a decarboxylation enzyme, arylmalonate decarboxylase (AMDase, EC 4.1.1.76).[5] AMDase has been isolated as a unique decarboxylase to catalyze the decarboxylation of malonates to give optically pure α-arylpropionates (Scheme 1). In addition to this original activity, we have found that the enzyme has aldolase activity to catalyze the aldol-type condensation of the malonate derivative containing an aldehyde group.

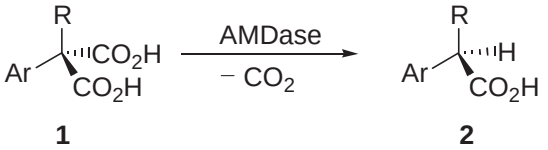
AMDase was purified from *Alcaligenes bronchisepticus* KU1201, which was isolated from soil. The gene coding the enzyme has already been cloned and overexpressed in *E. coli*, and the enzyme has also been purified.[6] The reaction mechanism of AMDase has been extensively studied. It has been clarified that Cys188 is located in the active site as the key amino acid residue, of which role has been revealed to protonate to the intermediate enolate form of the resulting carboxylic acid.

Recently, we have succeeded to endow AMDase with a new catalytic activity, i.e., racemization of carboxylic acids, in addition to its original decarboxylation activity. It was achieved by introducing only one mutation in the active site based on the estimated reaction mechanism and the homology with some isomerases.[7] Thus, it could be said that AMDase also had catalytic promiscuity similarly to lipases and other enzymes. In this case, the catalytic promiscuity of AMDase became apparent via the introduction of the mutation into the enzyme itself, i.e., by the modification of the enzyme. Then, we made another challenge to draw its catalytic promiscuity by designing the structure of the substrate. To trap the intermediate enolate resulting from the decarboxylation of the malonate, an aldehyde group was introduced at the ortho-position of the aromatic ring.

First, we tried the reaction of compound **3** expecting to obtain the condensation product **4**, which had six-membered ring (Scheme 2). However, no reaction occurred in this case, probably because the steric bulkiness of the substrate is too large.

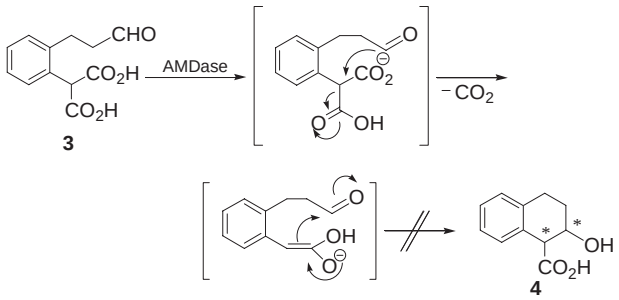
Then, we tried to synthesize a less bulkier substrate **14**, which was expected to give the condensation product with a five-membered ring. The substrate **14** was synthesized via nine steps from the commercially available compound **5** as illustrated in Scheme 3.

As expected, the treatment of **14** with AMDase (reaction conditions: 1.0% substrate, enzyme 100 unit, in 1.0 mL of 0.1 M Tris-HCl buffer, at 35 °C, for 12 h) gave the aldol product in 35% yield (Scheme 4). The product was identified by comparing the NMR and IR spectra with those of the authentic specimen. Because the reaction was taken place in the active site of the enzyme that generally differentiates the chirality and prochirality of the compounds, we expected that the aldol product would exhibit some enantio- or diastereomeric excess. However,



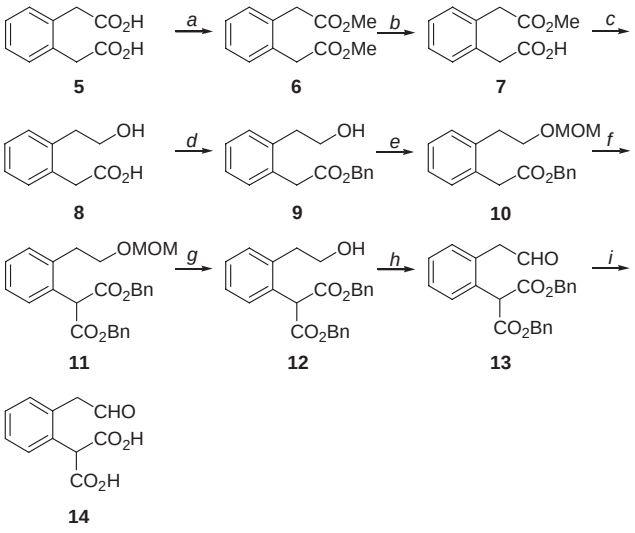
1 **2**

Scheme 1. Asymmetric decarboxylation of AMDase.



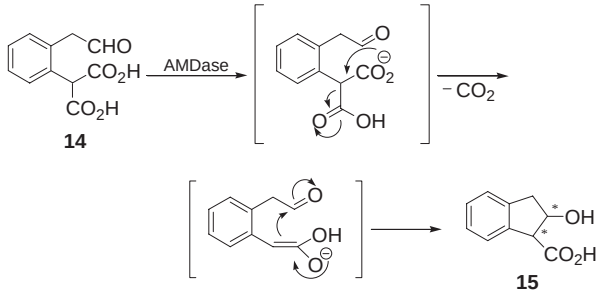
3 **4**

Scheme 2. Enzymatic aldol reaction catalyzed with AMDase.



Reagents *a*: H^+, MeOH, *b*: KOH, MeOH, H_2O, *c*: $LiBH_4$, THF, *d*: Cs_2CO_3 BnBr, DCM, *e*: MOMCl, *i*-Pr_2EtN, DCM, *f*: LDA, $ClCO_2Bn$, THF, *g*: Me_3SiBr, DCM, *h*: IBX-Resin, DCM, *i*: H_2, Pd/C, EtOH.

Scheme 3. Preparation of the substrate.



Scheme 4. Enzymatic aldol reaction catalyzed with AMDase.

unfortunately, the product was revealed to have neither ee nor de based on the HPLC and NMR spectra comparing with those of the authentic stereoisomers.[8]

The stereochemical selectivity of enzymatic reactions is generally determined by the steric relationship between the substrate and the amino acid residues of the enzyme. In the present case, AMDase showed no stereoselectivity, although the reaction is surely an event in the active site of the enzyme, because the starting material was recovered in the absence of the enzyme. The lack of the stereoselectivity is estimated to come from the structure of the substrate. As the aldehyde moiety and resulting enolate moiety are connected via a benzene ring, they are considered to occupy almost planar conformation with each other. In this situation, if the enzyme allows the aldehyde group a small deviation to both above and under the planar face, then the product will be a mixture of stereoisomers.

In conclusion, we have succeeded to obtain the aldol condensation product which is formed via the reaction of the enol intermediate resulting from the decarboxylation of the substrate, although the expected steric selectivity was not observed. Thus, AMDase has been demonstrate to have another catalytic promiscuity, in addition to racemase activity.[7] In contrast to the fact that the previous catalytic activity was achieved by the modification of the enzyme itself, the second catalytic activity was shed light by subjecting the well-designed substrate, which has an aldehyde group close to the intermediate enolate functionality.

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