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A new binding mode expands the substrate scope of Aryl Malonate Decarboxylase (AMDase)

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PURPOSE OF THE ABSTRACT
Precise control of enantioselectivity is a critical feature of biocatalysts for many industrial applications. Bacterial aryl malonate (AMDase) is a cofactor-free enzyme that generates a wide spectrum of optically pure carboxylic acids, including several with anti-inflammatory activity such as flurbiprofen. AMDase cleaves the pro-R carboxylate of arylaliphatic malonic acids, followed by stereoselective protonation of a planar intermediate by a catalytic cysteine giving rise to aryl methyl and vinyl methyl carboxylic acids in high optically purity. Wildtype AMDase with C188 produces the pure (R)-products under inversion of the stereocenter. AMDase (V43I/G74C/A125P/V156L/M159L/C188G) (CGLIPL) has the catalytic cysteine allocated at the other side of the planar intermediate and gives rise to the (S)-products under retention of the configuration. The substrate scope of AMDase is limited to substrates bearing an alpha-substitutent not larger than a methyl group, presumably due to the steric hindrance in a small hydrophobic pocket in the active site of the decarboxylase.

It was an unexpected finding that AMDase CGLIPL decarboxylated 2-methyl-2-vinyl malonic acid with low selectivity, giving rise to the final (S)-2-methyl-but-3-enoic acid with 66% ee [1]. As a possible explanation, an inverse binding mode resulting in the cleavage of the pro-S carboxylate presented itself, Scheme 1. We assumed that this binding mode should also allow the synthesis of carboxylic acids with larger alpha-substitutents, which would be a significant expansion of the substrate scope of the enzyme.

Here we probe the activity of the enigmatic AMDase CGLIPL variant. Using a newly synthesized pro-R-13C-labelled vinyl methyl malonate, we identified the leaving group (pro-R (13C) or pro-S carboxylic acid). The analysis of the stereochemical pathways indicated that the protonation after decarboxylation of 2-methyl-2-vinyl malonate in two different binding modes proceeds exclusively under inversion of the stereocenter. Surprisingly, retention of the stereochemistry was not observed.
We will show an analysis of the stereochemical pathways of AMDase and discuss mechanistic consequences. Furthermore, we will show how this novel binding mode unlocks the conversion of ethyl-substituted substrates with outstanding selectivity.

FIGURES

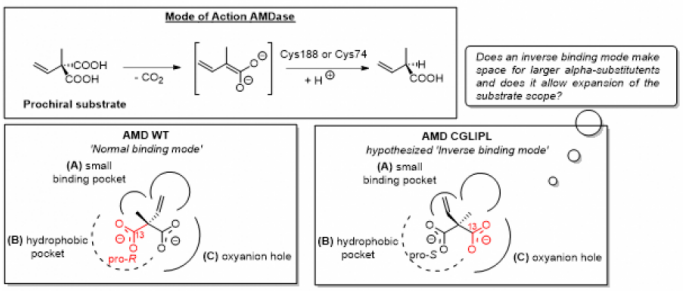

FIGURE 1

AMD binding modes

Scheme 1. Mode of action of AMDase, and binding of 2-methyl-2-vinyl malonate in AMD WT and CGLIPL variant.

FIGURE 2

KEYWORDS

Biocatalysis | Mechanism | Probe

BIBLIOGRAPHY

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