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Crystal Structure and Expanded Substrate Spectrum of the α-keto acid C-Methyltransferases SgvM and MrsA

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PURPOSE OF THE ABSTRACT

S-adenosylmethionine (SAM)-dependent methyltransferases (MTs) are involved in the C-methylation of a variety of natural products such as DNA, proteins, or small molecules.[1] Enzymatic C-methylation requires activation of the substrate's carbon atom by an adjacent functional group to form a nucleophilic intermediate carbanion and to enable nucleophilic attack on the methyl residue of SAM. A few examples of C-MTs have been described that methylate enolizable β-branched α-keto acids, leading to precursors of non-proteinogenic amino acids.[2] The MTs SgvM from Streptomyces griseoviridis and MrsA from Pseudomonas syringae pv. syringae catalyze the methylation of the β-carbon atom of α-keto acids in the biosynthesis of the antibiotic natural products viridogrisein and 3-methylargenine, respectively.[3] MrsA shows high substrate selectivity for its native substrate 5-guanidino-2-oxovalerate, while other α-keto acids, such as the SgvM substrates 4-methyl-2-oxovalerate, 2-oxovalerate, and phenylpyruvate, were not accepted.

Here, we report on the crystal structures of SgvM and MrsA in the apo form, in complex with their substrates, SAM, and methyladenosine, a degradation product of SAM, respectively. By investigating key substrate recognition residues in the active site of both enzymes and through site-directed mutagenesis, the substrate spectrum of MrsA was extended to accept the α-keto acid substrates of SgvM with uncharged and lipophilic β-residues. Our results showcase the possibility to transfer the substrate promiscuity of α-keto acid MTs from distinct biosynthetic pathways by rational design.

FIGURES

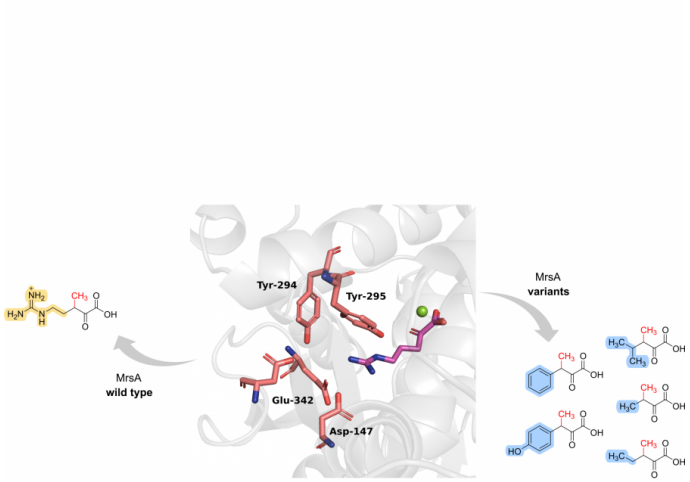

FIGURE 1

Expanded Substrate Spectrum of MrsA

Active site of MrsA wild type in complex with its native substrate 5-guanidino-2-oxovalerate (dark grey) and Mg2+ ion (green). The residues of the mutation sites for MrsA variants are highlighted in red.

FIGURE 2

KEYWORDS

C-methyltransferase | α-keto acid | crystal structure | site-directed mutagenesis

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