α,α-DISUBSTITUTED α-AMINO ACID METABOLISM INCLUDING A NOVEL THREE-COMPONENT NON-HEME DIIRON MONOOXYGENASE SYSTEM

Jun Ogawa, Div. Appl. Life Sci., Grad. Sch. Agric., Kyoto Univ., Kyoto, Japan
ogawa.jun.8a@kyoto-u.ac.jp
Ryotaro Hara, Lab. Ind. Microbiol., Grad. Sch. Agric., Kyoto Univ., Kyoto, Japan
Michiki Takeuchi, Lab. Ind. Microbiol., Grad. Sch. Agric., Kyoto Univ., Kyoto, Japan
Makoto Hibi, Biotechnol. Res. Center & Dep. Biotechnol., Toyama Prefectural University, Toyama, Japan

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The high-valent iron-oxo species formed in the non-heme diiron enzymes have high oxidative reactivity and catalyze difficult chemical reactions. Although the hydroxylation of inert methyl groups is an industrially promising reaction, utilizing non-heme diiron enzymes as such a biocatalyst has been difficult. Here we show a three-component monooxygenase system for the selective terminal hydroxylation of α-aminoisobutyric acid (Aib) into α-methyl-D-serine (D-MeSer).

Aib is one of the extraterrestrial amino acids contained in the sample brought back by Hayabusa-2 from the asteroid Ryugu. Aib is also a prochiral substrate generating D-MeSer, a pharmaceutical synthesis intermediate, via stereoselective hydroxylation. During the bioprocess development to produce D-MeSer from Aib, we found *Rhodococcus wratislaviensis* C31-06 strain that metabolizes Aib with D-MeSer as an intermediate. We also identified Aib hydroxylase, which catalyzes the initial reaction of Aib metabolism in this strain, as a three-component non-heme diiron monooxygenase system. It consists of the hydroxylase component, AibH, and the electron transfer component (AibF and AibG). The crystal structure analysis revealed that AibH forms a heterotetramer of two amidohydrolase superfamily proteins (AibH1 and AibH2), of which AibH2 is a non-heme diiron protein and functions as a catalytic subunit. The Aib monooxygenase was demonstrated to be a promising biocatalyst that is suitable for bioprocesses in which the inert C–H bond in methyl groups need to be activated[1)].

We analyzed the proteins encoded in the gene cluster containing Aib hydroxylase (Aib gene cluster) and found a variety of novel enzymes. The genes *aibF* and *aibG*, located near the Aib hydroxylase genes (*aibH1* and *aibH2*), encode FMN-dependent ferredoxin reductase and Rieske-type ferredoxin, which transfer electrons from NADH to the hydroxylase, respectively. In addition, analysis of a series of enzymes encoded by the Aib gene cluster revealed that D-MeSer is converted to 2-amino-2-methylmalonic acid semialdehyde by AibD1 (NAD^+-dependent alcohol dehydrogenase) and further to 2-amino-2 methylmalonic acid (Amm) by AibE (NAD^+-dependent aldehyde dehydrogenase). Amm was converted to L-alanine by a novel enzyme, AibC, catalyzing Amm decarboxylation in the presence of PLP (Figure 1).

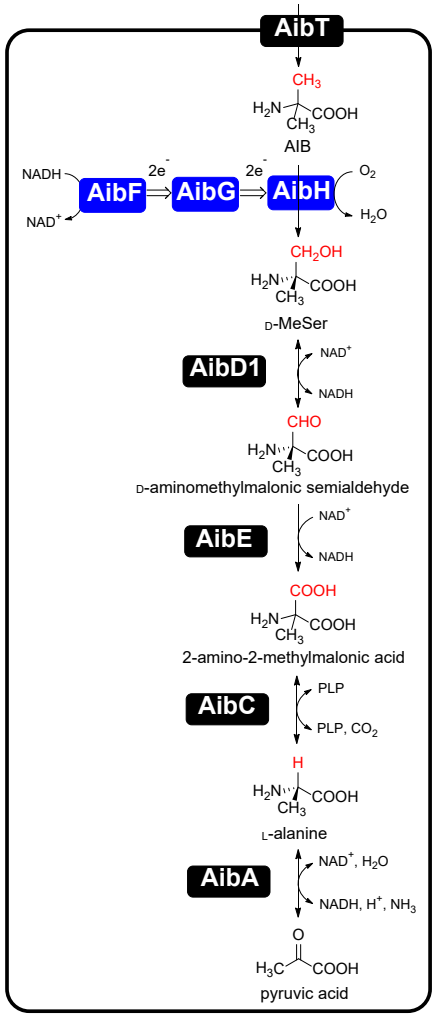

Figure 1 Aib metabolism in Rhodococcus wratislaviensis C31-06

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