Hydroxylation of Nonnative Substrates by Wild-type Cytochrome P450BM3 with Decoy Molecules

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Cytochrome P450BM3 (P450BM3) is one of the most promising P450s as potential biocatalysts for applications in green synthetic chemistry, as they possess high activity for the hydroxylation of inert substrate C–H bonds. Because the substrate-binding is crucial for the generation of active species of P450BM3 (Compound I), substrates whose structures are largely different from that of its native substrates (long-alkyl-chain fatty acids) cannot be hydroxylated by P450BM3 (Figure 1). To enable oxidation of non-native substrates by P450BM3 without any mutagenesis, we have developed a series of "decoy molecules", inert dummy substrates, with structures that resemble those of the native substrates. Decoy molecules fool P450BM3 into generating Compound I, enabling the catalytic oxidation of non-native substrates other than fatty acids (Figure 1). Perfluorinated carboxylic acids (PFCs) serve as decoy molecules to initiate the activation of molecular oxygen in the same manner as long-alkyl-chain fatty acids, due to their structural similarity, and induce the generation of Compound I, but, unlike the native substrates, PFCs are not oxidizable by Compound I, allowing the hydroxylation of non-native substrates, such as gaseous alkanes and benzene (Figure 1).[1] The catalytic activity for non-native substrate hydroxylation was significantly enhanced by employing 2nd generation decoy molecules, PFCs modified with amino acids (PFC-Amino acids). The crystal structure analysis of P450BM3 with the 2nd decoy molecule (*N*-perfluorononanoyl-*L*-tryptophan:PFC9-Trp) showed that the decoy molecule binds to the substrate access channel of P450BM3. Recently, we have demonstrated that various amino acids (*N*-acyl amino acids), as well as amino acid dimers having a completely different structure from fatty acids (3rd generation decoy molecules), can serve as decoy molecules. Benzene was more efficiently hydroxylated in the presence of these decoy molecules.[2, 3] We also have confirmed that wild-type P450BM3 expressed in *E.coli* can be activated by adding amino acid derivatives as decoy molecules to the culture medium, and benzene was hydroxylated without supplementing with NADPH.[4] Activities of the whole-cell biocatalyst drastically varied depending on the structure of decoy molecules added to the cell suspension, suggesting that difference in permeability between decoy molecules may affect the activation of intracellular P450BM3.[5] We have succeeded in further enhancing the catalytic activity for benzene and gaseous alkane hydroxylation by systematic screening of decoy molecules.[6] Furthermore, we have found that one of the decoy molecules can accelerate the crystallization of P450BM3 and reported crystal structures of the various flavour of P450BM3.[7]

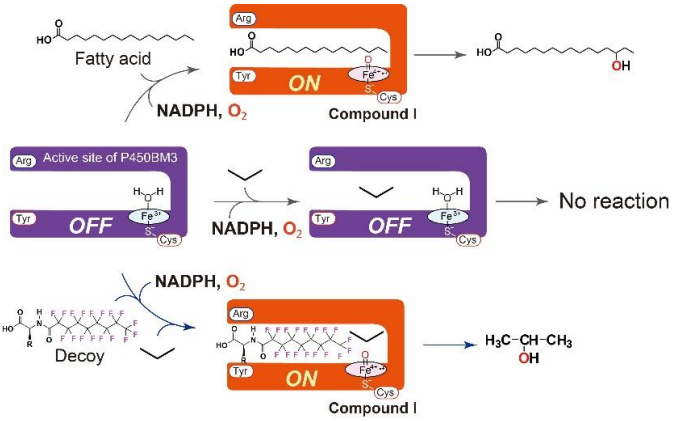

Figure 1. Schematic representation of fatty acid hydroxylation (upper) and propane hydroxylation in the presence of a decoy molecule (bottom) catalyzed by P450BM3. The reaction of propane in the absence of any decoy molecule (middle).

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