Gaseous Alkane and Benzene Hydroxylation by Cytochrome P450BM3 with Decoy Molecules

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Abstract: Cytochrome P450BM3 (P450BM3) is a heme enzyme and catalyzes hydroxylation of long-alkyl-chain fatty acids. 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 cannot be hydroxylated by P450BM3. We found that P450BM3 starts to catalyze hydroxylation of nonnative substrates in the presence of perfluorinated carboxylic acids (PFCs) and *N*-perfluoroacyl amino acids as inert dummy substrates (decoy molecules). Decoy molecules initiate the activation of molecular oxygen in the same manner as with long-alkyl-chain fatty acids and induce the generation of compound I, but the compound I hydroxylates gaseous alkanes and benzene because the C–F bonds of PFCs are not oxidizable.

Keywords: Cytochrome P450, Gaseous Alkane, Decoy Molecule.

1. Introduction

Enzymes catalyze a wide variety of reactions under very mild conditions, and have therefore been regarded as promising green catalysts for producing pharmaceuticals, fine chemicals, and biofuels. Developing a catalyst for selective oxyfunctionalizations is a longstanding challenge and a current topic of interest. Cytochrome P450s (P450s) are regarded as potential candidates for the development of biocatalysts because of their high catalytic activity in the hydroxylation of unactivated C–H bonds. Among the reported P450s, CYP102A1 (P450BM3) isolated from *Bacillus megaterium* has garnered much attention because of its high monooxygenase activity. In general, P450BM3 displays a high substrate specificity, exclusively catalyzing the hydroxylation of long-alkyl-chain fatty acids (Fig. 1a) while remaining inactive for small non-native substrates such as propane and benzene. However, it was observed that P450BM3 can be "fooled" into initiating hydroxylation of non-native substrates in the presence of perfluorinated carboxylic acids (PFCs), which function as inert dummy substrates (decoy molecules). PFCs initiate the activation of molecular oxygen in the same manner as with long-alkyl-chain fatty acids and induce the generation of compound I, but the compound I hydroxylates gaseous alkanes and benzene because the C–F bonds of PFCs are not oxidizable (Fig. 1a).[1)]

2. Experimental

Procedure for gaseous alkane hydroxylation: To a stirred gas-saturated buffer solution (propane/oxygen 80/20 v/v) in a glass sample bottle at room temperature were successively added P450BM3 (0.5 μM), a methanolic solution of decoy molecule (100 μM), and NADPH (5 mM). The reactor was immediately fitted with a balloon (gaseous alkane/oxygen ca. 1/1), and air in the bottle was replaced by the mixed gas for 2 min. After addition of NADPH to start the reaction, the reaction was carried out for 10 min, and then a sample of the reaction mixture (10 μL) was taken to determine the consumption of NADPH. The

obtained products were analyzed by gas chromatography (GC2014; Shimadzu) with an Rtx-1 column (Restek).

3. Results and discussion

We have succeeded in developing the next generation of decoy molecules by modifying the carboxylate of PFCs with amino acids and succeeded in enhancing the catalytic activity for gaseous alkanes. Amongst the next generation of decoy molecules examined, *N*-perfluorononanoyl-*L*-leucine (PFC9-*L*-Leu) was the most effective for hydroxylation of propane (256/min/P450) and ethane (45/min/P450).[2)]Furthermore, we have succeeded in crystallizing the *N*-perfluorononanoyl-*L*-tryptophan (PFC9-*L*-Trp)-bound form of P450BM3(Fig. 1b). The crystal structure analysis of PFC9-*L*-Trp-bound form of P450BM3 (PDB code: 3WSP) showed that the terminal of alkyl chain does not reach to the active site owing to the multiple hydrogen bonding interactions between the carboxyl and carbonyl groups of PFC9-*L*-Trp and amino acids (Tyr-51, Gln-73, and Ala-74) located at the entrance of P450BM3 (Fig. 1b). More recently, we have demonstrated that various carboxylic acids modified with amino acids (*N*-acyl amino acids) as well as amino acid dimers having a completely different structure from fatty acids can serve as decoy molecules.[3)]

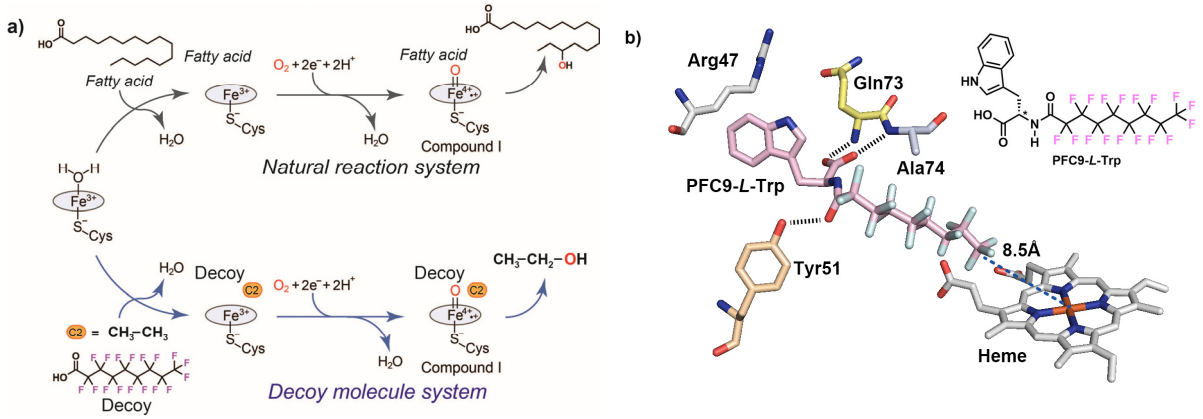

Figure 1. a) General reaction mechanism of fatty acid hydroxylation catalyzed by P450BM3 (upper) and a plausible reaction mechanism of ethane hydroxylation catalyzed by P450BM3 with perfluorononanoic acid (PFC9) as a decoy molecule (lower). b) The active site structure of P450BM3 with *N*-perfluorononanoyl-*L*-tryptophan (PFC9-*L*-Trp).

4. Conclusions

We have demonstrated that *N*-perfluoroacyl amino acids strongly activate wild-type P450BM3 for the hydroxylation of inert alkanes. PFC9-*L*-Leu gave PFRs for the hydroxylation of secondary and primary C−H bonds of alkanes that were comparable with those of the best P450 variants prepared by multiple-round mutagenesis. The crystal structure of the PFC9-*L*-Trp-bound form of P450BM3 revealed the active site re-formation and provided mechanistic insight into the activation of wild-type enzyme by decoy molecules. We conclude that the catalytic activity for gaseous alkane hydroxylation by wild-type P450BM3 would be improved further by optimizing the structure of the decoy molecule.

References

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