Analysis of Activation Mechanisms of Cytochrome P450BM3 by Decoy Molecules

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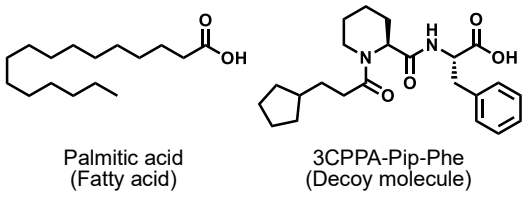
Cytochrome P450BM3 (P450BM3) is a heme enzyme which exhibits the highest monooxygenase activity toward long chain fatty acids among reported P450s while the enzyme does not hydroxylate non-native substrates such as benzene. However, we achieved benzene hydroxylation catalyzed by wild-type P450BM3 by adding amino acid derivatives.[1] We named such functional molecules “decoy molecules.” Decoy molecules activate P450BM3 by binding to P450BM3 in a similar manner to native substrates. However, decoy molecules themselves are not hydroxylated because of shortage of chain length. The small reaction space for non-native substrate hydroxylation is therefore formed at the catalytic site. The structure of decoy molecules has been improved to enhance catalytic activity of P450BM3. Recently, we demonstrated that the systematic screening of dipeptide derivatives is effective way to discover more effective decoy molecules in benzene hydroxylation.[2] However, activation mechanism in the reaction system is still unclear.

Herein, we performed isothermal titration calorimetry (ITC) analysis of the binding of ligands such as fatty acids and decoy molecules to P450BM3 to discuss the difference of the binding between fatty acids and the decoy molecules developed by the screening. We utilized liposome-bound ligands for the analysis because the ligands are insoluble to buffer due to their hydrohpobicity.[3] The thermodynamic parameters indicates that the binding of both fatty acids and decoy molecules (Figure 1) is entropy driven (Figure 2). From the results of the ITC analysis and other data, we discuss the activation mechanisms of P450BM3 by decoy molecules.



Palmitic acid (Fatty acid)　　3CPPA-Pip-Phe (Decoy molecule)

Figure 1. Structures of the typical fatty acid and decoy molecule

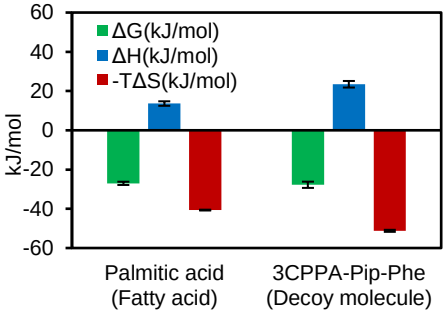


Figure 2. Thermodynamic parameters in the binding of ligands to P450BM3

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