

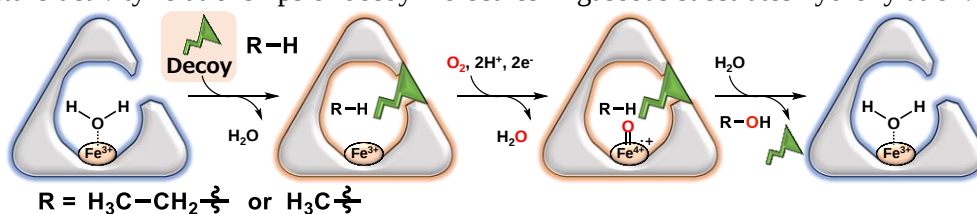
Screening of Peptide Derivatives for the Activation of Wild-Type Cytochrome P450BM3 for Gaseous Substrates Hydroxylation

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The monooxygenase cytochrome P450BM3 (P450BM3) is a heme enzyme which catalyzes hydroxylation of long chain fatty acids at an extremely high rate. Despite its advantageous catalytic activity, P450BM3 does not catalyze hydroxylation of non-native substrates such as benzene due to its high substrate specificity. However, we have 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 (Figure). The structure of decoy molecules has been improved to enhance catalytic activity of P450BM3. Recently, we demonstrated that screening of dipeptide derivatives is effective way to discover more effective decoy molecules in benzene hydroxylation.²

Herein, we adopted systematic screening strategy to examine hydroxylation activity of bromoethane and bromomethane catalyzed by P450BM3 in the presence of decoy molecules. Haloalkanes were chosen as surrogate substrates of gaseous alkanes for easy detection of aldehydes formed after α -elimination of HBr upon hydroxylation by colorimetric purpald assay.³ Previously reported decoy molecule library (containing over 160 molecules) and newly prepared library (containing over 20 molecules) were screened. Through the screening, specialized decoy molecules for haloalkanes hydroxylation by wild-type P450BM3 were discovered. Moreover, detectable amount of ethanol and methanol by GC-MS analysis was formed after catalytic ethane and methane hydroxylation under high pressure. Furthermore, the catalytic activity was improved using novel decoy molecules with mutated P450BM3. Based on the results of the screening, we discuss the structure-activity relationships of decoy molecules in gaseous substrates hydroxylation.



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