Molecular Design of Oxygenases Applicable to Synthetic Chemistry

Yoshihito Watanabe

(Nagoya University, Research Center for Materials Science, Professor)

【Outline of survey】

Cytochrome P450 is an attractive monooxygenase which is able to oxidize less reactive hydrocarbons to afford alcohols. Unfortunately, P450 consumes a stoichiometric amount of very expensive NAD(P)H due to the reductive activation of molecular oxygen. While $P450_{BS\beta}$ is known to oxidize fatty acid by using H_2O_2, it exclusively oxides fatty acid having alkyl chain length more than 10. In order to apply the P450 functions to synthetic processes, we have developed a series of myoglobin mutants, which utilize H_2O_2 for highly enantioselective sulfoxidation and epoxidation. In this research project, we focus on the following three subjects:

1) Construction of robust heme enzymes under the catalytic conditions.
2) Introduction of substrate versatility into $P450_{BS\beta}$.
3) Introduction of substrate recognition sites into myogobin mutants.

【Expected results】

Molecular design and construction of metalloproteins are very challenging subjects in the field of chemical biology as well as bioinorganic chemistry. Through the project, we will design and construct heme enzymes which are able to proceed hydroxylation showing high catalytic activities as well as high stereo- and enantioselectivities.

【References by the principal investigator】

・O. Shoji, T. Fujishiro**,** H. Nakajima, M. Kim, S. Nagano, Y. Shiro, and Y. Watanabe, "Hydrogen Peroxide Dependent Monooxygenations by Tricking the Substrate Recognition of Cytochrome $P450_{BS\beta}$" *Angew. Chem. Int. Ed*., **46**, 3656-3659, 46 (2007).

・T. Ueno, N. Yokoi, M. Unno, T. Matsui, Y. Tokita, M. Yamada, M. Ikeda-Saito, H. Nakajima, and Y. Watanabe, *"Design of Metal Cofactors Activated by Protein-Protein Electron Transfer System*." *Proc. Natl. Acad. Sci. USA* **103**, 9416-9421 (2006).

・T.D. Pfister, T. Ohki, T. Ueno, I. Hara, S. Adachi, Y. Makino, N. Ueyama, Y. Lu, and Y. Watanabe, "*Monooxygenation of an Aromatic Ring by F43W/H64D/V68I Myoglobin Mutant and Hydrogen Peroxide*," *J. Biol. Chem*. **280**, 12858-12866 (2005).

・Y. Watanabe and T. Hayashi, "Functionalization of Myoglobin," in *Progress in Inorganic Chemistry*, vol. 54 (ed., K.D. Kirlin), 449-493 (Wiley) (2005).

【Term of project】 FY2007−2011	【Budget allocation】 29,300,000 yen (2007 direct cost)

【Homepage address】 http://bioinorg.chem.nagoya-u.ac.jp