Substitutions of Artificial Amino Acids O-Methyl-Thr, O-Methyl-Asp, S-Methyl-Cys, and 3-Amino-Ala for Thr-252 of Cytochrome P-450<sub>cam</sub>: Probing the Importance of the Hydroxyl Group of Thr-252 for Oxygen Activation

Yoko Kimata<sup>1</sup>, Hideo Shimada<sup>1</sup>, Tada-aki Hirose<sup>2</sup>, and Yuzuru Ishimura<sup>1</sup>

Summary. In the monooxygenation reaction catalyzed by cytochrome P-450<sub>cam</sub> (P-450<sub>cam</sub>), Thr-252, a hydroxyl amino acid at the active site, is essential for the reductive cleavage of the O–O bond of oxygen: the hydroxyl (OH) group of Thr has been proposed to serve as an acid catalyst for the O–O bond scission. In this study, four different artificial amino acids were incorporated into the 252-position to verify the role of the OH group. The catalytic activities of the mutant enzymes suggest that the OH group does not function as the catalyst. Then, we propose that the OH group serves as an anchor of an acid catalyst and facilitates its catalytic action to cleave the O–O bond; water is possibly the catalyst.

Key words. Artificial amino acid—Mutagenesis—Cytochrome P-450—Catalytic mechanism—Hydrogen bond

Introduction

Cytochrome P-450<sub>cam</sub> (P-450<sub>cam</sub>) in Pseudomonas putida is a heme-containing monooxygenase that catalyzes the reaction: d-camphor + NADH + H<sup>+</sup> + O<sub>2</sub> $\Rightarrow $ 5-exo-hydroxycamphor + NAD<sup>+</sup> + H<sub>2</sub>O. In this reaction, the O–O bond of oxygen is cleaved; one of the oxygen atoms is incorporated into d-camphor, and the other atom is converted to H<sub>2</sub>O. The mechanism of dioxygen scission to form an active oxygen species that monooxygenates substrate is one of the major questions in the reaction catalyzed by P-450<sub>cam</sub> as well as other heme-containing monooxygenases. At the oxygen scission step, two protons are required. Thr-252 has been proposed to participate in a proton delivery system (Fig. 1) through a hydrogen-bonding network (Thr-252–H<sub>2</sub>O–Asp-251-Lys-176/Arg-186) extending from the interior active site to the outside of the protein [1–5]. In this system, the OH group of Thr-252 is an acid catalyst for the oxygen scission that donates a proton to the heme-bound oxygen: the OH group can act as a donor and acceptor of the hydrogen bond, which property is required for the

<sup>1</sup>Department of Biochemistry, <sup>2</sup>Pharmaceutical Institute, Keio University, 35 Shinanomachi, Shinjuku-ku, Tokyo 160, Japan

Y. Ishimura et al. (eds.), Oxygen Homeostasis and Its Dynamics © Springer-Verlag Tokyo 1998