Origin of Functional Difference in Structurally Homologous Enzymes, Coral Allene Oxide Synthase and Catalase; Resonance Raman Investigation

Takehiko Tosha[1], Takeshi Uchida[1], Alan R. Brash[2] and Teizo Kitagawa[1]

[1]*Okazaki Institute for Integrative Bioscience, National Institutes of Natural Sciences, and* [2]*Department of Pharmacology, Vanderbilt University Medical Center.*

Allene oxide synthase for the eicosanoid biosynthesis in coral is consisted of heme-containing allene oxide synthase (cAOS) and 8*R*-lipoxygenase domains. The crystal structure of cAOS revealed that the heme environments including the proximal tyrosinate ligand are largely conserved between cAOS and catalase. cAOS, however, catalyzes the formation of an allene oxide from fatty acid hydroperoxide, and shows no catalase activity. Here, for the purpose of understanding the origin of the functional difference between cAOS and catalase, we examined the reaction of cAOS with H_2O_2 by using stopped flow method and clarified which step in the catalase reaction is inhibited in cAOS. Also we characterized the structure of the heme environment in cAOS in comparison with that of catalase by resonance Raman spectroscopy.

In contrast to catalase that forms the reaction intermediate such as compound I upon the addition of H_2O_2, no changes were detected in the optical spectrum for cAOS upon the addition of H_2O_2. We, therefore, conclude that cAOS cannot react with H_2O_2 to from compound I. To elucidate its reason, we examined resonance Raman spectra of the resting state to characterize the heme environment. The porphyrin marker lines showed that ferric cAOS has a 6-coordinate/high spin heme with water as a 6th ligand. Because the heme in catalase adopts a 5-coordinate/high spin state, the heme environments, especially distal site, are different between cAOS and catalase. The different environments in the heme distal site were also confirmed for the reduced state by lower $\nu_{Fe\text{-}CO}$ and higher $\nu_{C\text{-}O}$ for CO-bound cAOS than those in catalase. Based on the correlation between $\nu_{Fe\text{-}CO}$ and $\nu_{C\text{-}O}$, we suggest that the distal environment would be less positive and/or that the hydrogen bonding interactions between liganded CO and the surrounding residues would be weaker in cAOS than in catalase. These observations allow us to suggest that distal His and Asn, both of which are crucial for the cleavage of the O-O bond in H_2O_2 to form compound I in catalase, might not be located at the position interacting with the ligated CO, and thus to propose that the lack of the interactions between distal residues and H_2O_2 would cause no catalase activity in cAOS.