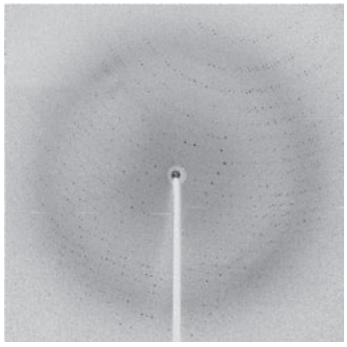
Clostridium acetobutylicum, an obligatory anaerobe. Rpr consists of 181 amino acids with a molecular weight of 22,500. It is rubrerythrin-like protein, and conserves rubredoxin-type [$Fe(SCys)_4$] site in the N-terminus. Rpr rapidly reduces hydrogen peroxide using NADH as an electron donor, and slowly reduces t-butyl hydroperoxide and dioxygen. $2H^+ + 2Rpr_{red}(1Rpr_{dimer\ red}) + H_2O_2 = 2Rpr_{ox}(1Rpr_{dimer\ ox}) + 2H_2O$ In order to reveal the reaction mechanism of the reduction of hydrogen peroxide by Rpr, we have started the crystallographic study. Purified Rpr was crystallized by the sitting-drop vapor diffusion method using poly(ethylenglycol)(PEG) 4000 as a precipitant (final composition of the droplet: 12 mg/mL in 50 mM K-Pi pH 7.0 buffer, Outer droplet: 9% PEG 4000, 0.1 M Zn acetate in 0.1 M MES pH 6.0 buffer). Crystals diffracted to 2.3 angstrom resolution at the beamline BL38B1 (SPring-8), and belong to the space group $P2_12_12$ (a = 81.6 Å, b = 117.4 Å, c = 141.5 Å). Structure analysis is now in progress.



Keywords: X-ray crystallography of proteins, metalloproteins, redox enzymes

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Roles of heme-6-propionate side chain in monooxygenase cytochrome P450cam

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Cytochrome P450cam (P450cam) responsible for *d*-camphor hydroxylation binds a protoheme IX as a prosthetic group in which two heme-propionate side chains interact with Arg112 and Arg299, respectively. To understand the structural and functional roles of the heme-6-propionate side chain in P450cam, we prepared the reconstituted P450cam with one-legged heme where the 6-propionate side chain was replaced with methyl group. The crystal structure of the reconstituted P450cam at a resolution of 1.55 angstrom is highly superimposable with that of the wild type protein, and a chloride anion is located in the position of the 6-propionate (Figure 1). The reactivity of the reconstituted P450cam toward the substrate hydroxylation was almost comparable with that observed for the wild type protein, whereas we found that the removal of the 6-propionate side chain accelerated the unfavorable conversion of the P450 active form into the inactive P420 species. In this presentation, we discuss the exact roles of the 6-propionate side chain in P450cam.

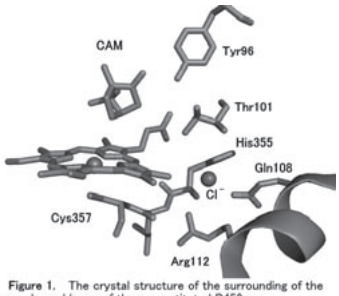

Figure 1. The crystal structure of the surrounding of the one-legged heme of the reconstituted P450cam.

[1] K. Harada, K. Sakurai, H. Shimada, T. Hayashi *et al.* (2007) *J. Am. Chem. Soc.*, **130**, 432.

Keywords: heme proteins, monooxygenases, crystallography of biological macromolecules

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The crystal structure of heme oxygenase catalytic intermediate unravel the enzyme mechanism

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Heme oxygenase (HO) catalyzes O_2-dependent regiospecific conversion of heme to biliverdin, CO, and free iron by three consecutive monooxygenase steps. In order to understand the reaction mechanism of HO, we have solved the structures of catalytic intermediate of HO catalysis using HmuO, a heme oxygenase from *Corynebacterium diphtheriae*. In the substrate-free structure, the proximal and distal helices, which sandwich the heme group, move farther apart with changes in their conformations resulting in opening of the heme pocket so as to fascilitate heme binding. Crystals of the hydroperoxo intermediate, have been prepared using cryogenic reduction technique, in which the oxy crystals were irradiated by synchrtron radiation at 100 K. The structure of the ferric hydroperoxo heme-HmuO complex is very similar to that of the oxy form but has slightly longer Fe-O and O-O bond distances. Flash-annealing of the irradiated oxy crystals has yielded the hydroxyheme intermediate, the structure of which has been solved and refined at high resolution. In the structure, the distal helix moves away from hydroxyheme. Possible reaction mechanism, based on the crystal structures of catalytic intermediates will be discussed at the meeting.

Keywords: heme enzyme structure and function, reactive intermediate, cryo crystallography

P4

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X-ray crystal structural analysis of cyanide binding cytochrome *c* oxidase

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For elucidation of the reaction mechanism of the cyotochrome *c* oxidase(CcO), it is desirable to determine the binding mechanism of cyanide to the oxygen reduction site of the enzyme. Here, we analyzed the structure of the cyanide derivative of the fully oxidized CcO. Cyanide induces extremely small change in the α-band spectrum of the enzyme. Thus, it is impossible to trace cyanide-binding to the enzyme in the crystals by measuring the absorption spectrum of the crystals, because accurate measurement of Soret-band of the enzyme in crystals is impossible. However, we found that cyanide once bound to CcO was not removal by repeat dialysis,