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High Resolution X-Ray Analysis of Peroxidase in Complex with Substrate Analog/Inhibitor

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Heme peroxidase catalyzes the two-electron oxidation of a variety of compounds by utilizing hydrogen peroxide [1]. The resting peroxidase reacts with hydrogen peroxide to give an intermediate called compound I, which is reduced to the resting state *via* the second intermediate, compound II, by the use of reducing substrates. Most substrates as well as inhibitors may bind to the distal side of the heme. In view of importance of accurate coordination geometry to the heme iron, we collected high resolution data of peroxidase from the fungus *Arthromyces ramosus* (ARP) in complex with substrate analog/inhibitor.

Crystals of ARP complexes were prepared by the soaking method as described elsewhere [2,3]. These crystals are tetragonal, space group $P4_22_12$ with one molecule per asymmetric unit. Flash-cooled crystals had cell-dimensions of a=b=73.8 Å, c= 115.8 Å.

Diffraction data were collected with λ=0.71 Å and MAR CCD deterctor at BL41XU. In order to cover wide intensity range, two sets of intensities recorded with long and short exposures were collected from each crystal. Data were processed and scaled in a standard way. Results of the data collection are shown in Table 1. Rrefinements using these data may provide more detailed structures than before. Preliminary refinement for the hydroxylamine complex revealed unexpected orientation of the hydroxylamine relative to the heme group.

References

1) K. Fukuyama, *Handbook of Metalloproteins*, pp.222-232, John Wiley & Sons (2001).
2) K. Fukuyama *et al. J. Biol. Chem.* **270**, 21884-21892 (1995).
3) H. Wariishi *et al., J. Biol. Chem.* **275**, 32919-32924 (2000).

Table 1. Results of data collection

	Native	NH_2OH	CN	NO
Oscillation angle (°)	1.0/3.0	0.8/2.4	1.0/3.0	0.8/3.0
Crystal-CCD distance (mm)	120	120	120	120
Resolution (Å)	1.4	1.3	1.3	1.3
No. of measurements	571,406	629,850	697,832	610,551
No. of unique reflections	62,946	78,980	78,588	78,992
Completeness (%)	98.6 (94.8)	99.4 (97.4)	99.3 (97.1)	98.3 (92.9)
R_{merge} (%)	5.7 (22.6)	6.8 (27.9)	6.6 (23.6)	6.6 (26.4)

The values in parentheses are for the highest resolution shell.

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X-ray Crystallographic Analyses of Bovine Rhodopsin and Its Early Photo-Intermediates

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X-ray crystallographic studies of the visual pigments have just started last year, with an exciting picture of rhodopsin from bovine rod outer segment membranes. Its ground state structure determined at 2.8 angstroms (PDB ID: 1F88) provided a new template, on which a number of previous studies on not only the visual pigments but also the rhodopsin family of G protein-coupled receptors (GPCR) could be assembled to inspire further experimental/theoretical approaches to unveil the molecular mechanisms of G protein-mediated signal transduction.

A remarkable feature regarding light signal detection in the rod cell is its extreme high sensitivity. The underlying molecular mechanisms are, 1) High quantum yield (~0.67) in the cis-trans photo-isomerization of the 11-*cis*-retinal chromophore 2) Efficient light energy storage (~65 %) and utilization to evoke large protein conformational change and 3) Competence of the active form of rhodopsin to activate thousands copies of G protein transducin. To understand how rhodopsin achieves these functions, x-ray structure determination of a series of photo-intermediates (Batho, Lumi, M I and M II) is the most powerful method.

The photo-intermediates of bovine rhodopsin in solution have been well characterized by both low-temperature spectroscopy and laser flash photolysis at room temperature. The photoreactions of rhodopsin detected by these two techniques are basically consistent with each other. As an initial step in our project on rhodopsin intermediates, we have collected, at BL41XU, 1) a higher resolution data set for the ground state and 2) 8 and 4 data sets for the photo-activated rhodopsin crystals corresponding to Batho and Lumi respectively. We found that these could be trapped under cryogenic conditions (100 – 145 K) as reported for rhodopsin in solution. To evaluate the content of these intermediates in the crystals, we have also carried out microspectroscopy outside SPring-8. The analyses of these data sets are currently in progress.

During the two runs for this proposal, x-ray was excellent and most of the planned experiments were completed without any significant problems of the beamline. Because we have neither observed any serious damage even after collection of three data sets (90 degrees each, 30 sec exposure per flame) from one crystal, further optimization of the x-ray intensity would be very much appreciated.