Preliminary Neutron Diffraction Studies of NADH-Cytochrome b_5 Reductase at J-PARC

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NADH-cytochrome b_5 reductase (b5R), a flavoprotein consisting of NADH- and FAD-domains, catalyzes electron transfer from the two-electron carrier NADH to the one-electron carrier cytochrome b_5. The reaction catalyzed by b5R plays a role in fatty acid synthesis, cholesterol synthesis, and xenobiotic oxidation as a member of the electron transport chain on the endoplasmic reticulum. We have already determined the crystal structures of both the fully reduced (at 1.68 Å resolution) and oxidized (at 0.78 Å resolution) forms of porcine liver b5R by X-ray crystallography [1], but, its detail mechanism, especially hydride/proton transfers and exact states of semiquinone, still remains unknown. The hydrogen information obtained by neutron crystallography will be essential for the real understanding of catalytic cycle of the b5R. In this research, we aim to determine the b5R structure of the oxidized form which is an initial state of the catalytic cycle.

For neutron diffraction experiments, we prepared large crystals with the size of almost 2 mm^3. Large crystals were transferred to cryo-protectant solution by stepwise soaking method, and then were flash-frozen in a cold nitrogen gas stream. In preliminary neutron experiment, we confirmed some diffraction spots above 1.4 Å resolution from a crystal with the size of 1.8 mm^3 under 100 K at BL03 (iBIX), MLF, J-PARC after 14 hours exposure at 300 KW accelerator power [Fig. 1]. Continuously, we will perform full-data collection at 2014A period.

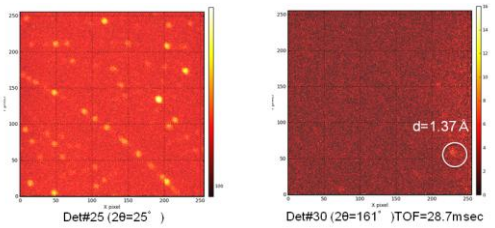

Fig. 1 Diffraction images of b5R collected at BL03, MLF, J-PARC.

References

[1] M. Yamada, T. Tamada, K. Takeda, F. Matsumoto, H. Ohno, M. Kosugi, K. Takaba, Y. Shoyama, S. Kimura, R. Kuroki, and K. Miki, J. Mol. Biol. **425**, 4296 (2013).