Crystallography

5A, NW12A/2007G210

X-ray crystal structure analysis of *A. suum* Complex II aimed for anti-parasitic drugs

Hironari SHIMIZU[2], Mayumi Shindou[1], Daniel Ken INAOKA[2], Yasutosi KIDO[2], Kazuaki MATOBA[1], Kiyoshi KITA[2], Shigeharu HARADA*[1]
[1]Graduate School of Science and Technology, Department of Applied Biology, Kyoto Institute of Technology, Kyoto 606-8585, Japan
[2]School of International Health, Graduate School of Medicine, The University of Tokyo, Tokyo 113-0033, Japan

Introduction

The anaerobic NADH-fumarate reductase system is found not only in nematodes but also in bacteria and many other parasites, and it is a promising target for chemotherapy [1]. The most potent inhibitor of Complex II, Atpenin A5, was found during screening of inhibitors for *A. suum* Complex II [2]. However, the mammalian Complex II is much more sensitive to Atpenin A5 than *A. suum* Complex II. On the other hand, we have found that flutolanil, a commercially available fungicide, specifically inhibited *A. suum* Complex II [3]. Therefore, we have taken flutolanil as a lead compound for structure-based drug design. In the current study, we purified, crystallized and performed X-ray structure analysis of *A. suum* Complex II in complex with flutolanil.

Result and Discussion

After solubilization from inner membranes using sucrose monolaurate as a detergent, the purified *A. suum* Complex II was crystallized in the presence of octaethyleneglycol dodecyl ether and dodecyl maltoside by the dialysis method using polyethylene glycol 3350 as a precipitant. Crystals of *A. suum* Complex II in complex with flutolanil were prepared by the soaking method.

X-ray diffraction data were collected at 100 K using synchrotron facilities, NW12A at Photon Factory (Tsukuba, Japan), by the rotation method. Data were processed and scaled using *HKL2000* and the diffraction data set to 2.90 Å resolution was obtained.

Based on the previously determined structure of *A. suum* Complex II (Fig. 1), the refined structure of *A. suum* Complex II in complex with flutolanil was obtained. *A. suum* Complex II consists of two hydrophilic subunits, the flavoprotein (Fp) subunit containing FAD and iron-sulfur cluster (Ip) subunit, and two hydrophobic membrane anchored cytochrome *b* subunits, CybL and CybS.

Flutolanil was located at the rhodoquinone binding pocket, which is formed at the interface formed by three subunits, Ip, CybL and CybS (Fig. 2). The pocket is formed by amino acid residues, which are conserved in Complex II from many species. Thus flutolanil seems to bind to both *A. suum* Complex II and mammalian Complex II through common interactions, such as cation-π interaction with an arginine residue and πH⋯π

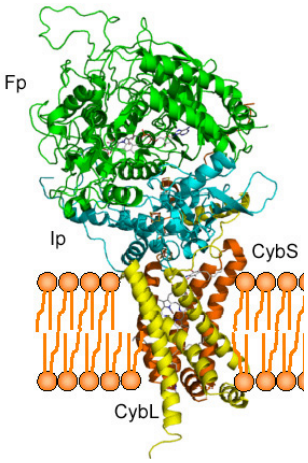

Fig. 1 The structure of *A. suum* QFR

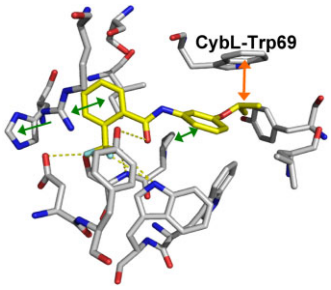

Fig. 2 The flutolanil binding site

interaction with a histidine residue (Fig. 2). However, CH⋯π interaction between flitolanil isopropyl group and Trp60 from CybL subunit is remarkable. Since Trp69 is an unique amino acid residue to *A. suum* Complex II and replaced with methionine in mammalian Complex II, the CH⋯π interaction would be one of the significant factors for the specific inhibitory effect of flutolanil to *A. suum* Complex II. In order to examine the specificity of flutolanil more detail, X-ray structure analysis of mammalian Complex II in complex with flutolanil is in progress.

References

[1] Omura, S., Miyadera, H., Ui, H., Shiomi, K., Yamaguchi, Y., Masuma, R., Nagamitsu, T., Iwai, Y., Takano, D., Sunazuka, T., Harder, A., Klbl, H., Namikoshi, M., Miyoshi, H., Sakamoto, K., & Kita, K. (2001) *Proc. Natl. Acad. Sci. USA* **98**, 60-62.
[2] Miyadera, H., Shiomi, K., Ui, H., Yamaguchi, Y., Masuma, R., Tomoda, H., Miyoshi, H., Osanai, A., Kita, K. & Omura, S. (2003) *Proc. Natl. Acad. Sci. USA* **100**, 473-477.
[3] Ito, Y., Muraguchi, H., Seshime, Y., Oita & Yanagi, S.O. (2004) *Mol. Gen. Genomics*, **272**, 328-335.

* harada@kit.ac.jp