CRYSTAL STRUCTURE OF FIXL, AN OXYGEN SENSING PROTEIN

Rhizobial FixL/FixJ comprise a two-component regulatory system which controls the gene expression of nitrogen fixation enzymes in response to O_2 concentration in plant root nodules. FixL contains a heme (Fe-protoporhyrin) in the sensor domain. Dissociation of O_2 from the heme moiety modulates the autophosphorylation activity of the kinase domain. FixJ acts as a transcriptional activator when it is phosphorylated by FixL and ATP. We carried out extensive structural and functional analyses to elucidate the O_2 sensing mechanism of FixL using X-ray crystallography [1].

We overexpressed the 150-residue oxygen sensor domain of *Rhizobium meliloti* FixL (RmFixLH). A single crystal of RmFixLH in the met (Fe^{3+}) form was grown in a 100 mM acetic acid/NaOH buffer at pH 4.6 in the presence of 200 mM ammonium acetate using 40% (w/v) PEG4000 as a precipitant. The crystals (0.2 mm × 0.05 mm × 0.01 mm) belong to the monoclinic space group (*C2*) with cell dimensions of *a* =60.94 Å, *b* =37.44 Å, *c* =54.14 Å and β =115.29°. X-ray diffractions were observed at beamline **BL44B2** up to a resolution of 1.1 Å (Fig. 1).

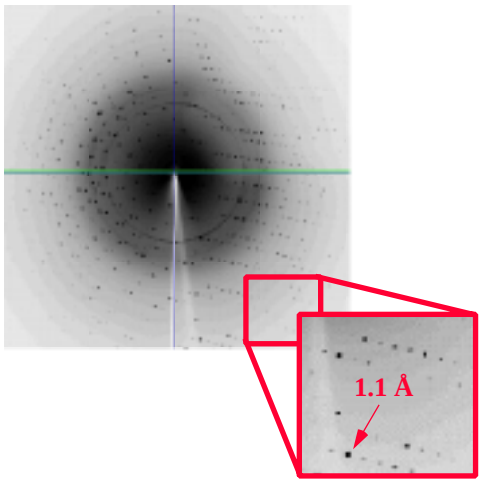

Fig. 1. X-ray diffraction image from a crystal of the RmFixLH at the atomic resolution (1.1 Å), measured by a Rigaku R-AXIS IV imaging plate detector.

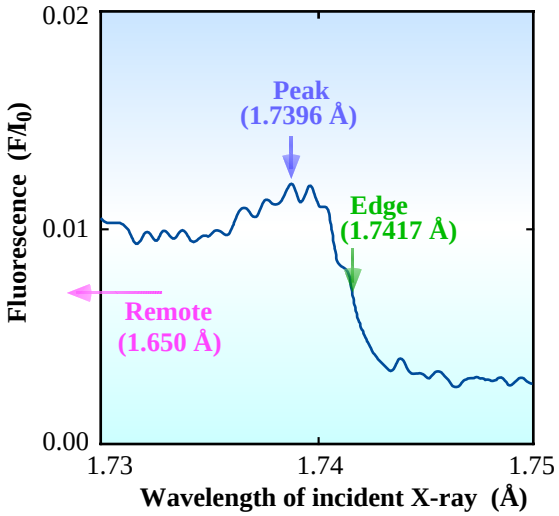

Fig. 2. XANES spectrum of Fe absorption in the RmFixLH crystal. The peak (1.7396 Å) and edge (1.7417 Å) are indicated by arrows.

Due to the lower solvent content in the crystal (22%), we were unable to prepare the isomorphous heavy atom derivatives. Thus, we carried out a MAD experiment using the anomalous scatter of the heme iron. The XANES spectrum for Fe was obtained (Fig. 2). Three data sets were collected at the X-ray wavelengths of 1.7396 Å (peak), 1.7417 Å (edge) and 1.6500 Å (remote) [2]. Only one crystal was used for the data collection. A valid peak corresponding to the iron clearly appeared in the Bijvoet difference Patterson map. We were able to fit the complete model for RmFixLH onto the electron density map calculated from the MAD phases (Fig. 3). We refined the model to 1.4 Å resolution, with R = 22% and R_{free} = 27%.

Figure 4 illustrates the overall shape of RmFixLH. The protein is ellipsoidal with dimensions of 50 Å × 35 Å × 35 Å. A crystallographic 2-fold axis exists along the dimerization interface (Helix II). Based on the crystal structure, we can explain the oxygen sensing mechanism of FixL. The O_2 binding site of

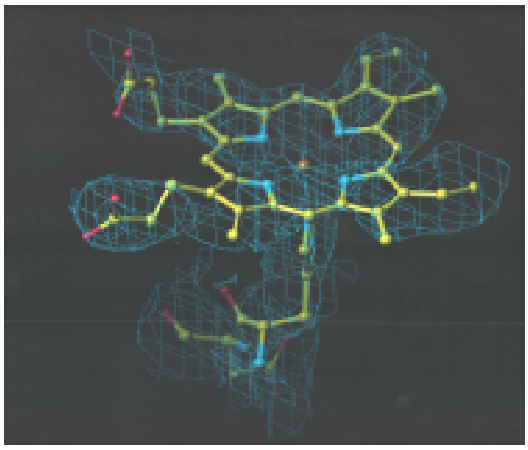

Fig.3. MAD phased initial electron density map of RmFixLH around the heme moiety at 2.4 Å resolution.

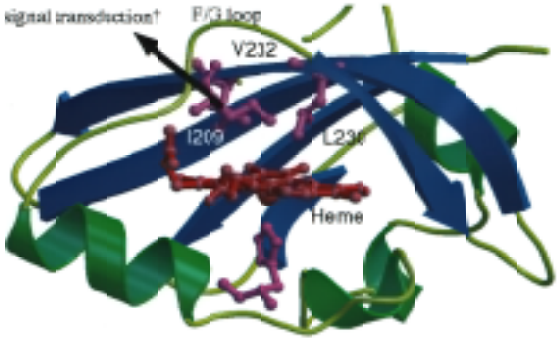

Fig. 5. Sensory center of RmFixLH. Three hydrophobic residues — Ile209, Leu230 — and Val232 are densely packed in the O_2 binding site. Ile209 is part of the flexible F/G loop region, and seems to be responsible for oxygen sensation.

RmFixLH is densely packed with three hydrophobic residues— Ile209, Leu230 and Val232 (Fig. 5). Steric repulsion should occur between the bound O_2 and these residues. Of the residues, Ile209 appears to be most responsive to the O_2 binding, because it is located in the most flexible loop (denoted as F/G loop in Fig. 5). Through a series of mutagenesis and spectroscopic studies, it was revealed that Ile209 was crucial for the sensor action [3]. We propose that the F/G loop changes its conformation when O_2 binds to the heme iron, which results in signal transduction from the sensory center to the histidine kinase domain [4]. We have already prepared the crystal of the CO-bound form of RmFixLH (analog of the O_2 -bound form), including the kinase domain of FixL. Upon determination of this structure in the near future, we will be able to explore the O_2 sensor mechanism in FixL in even more depth.

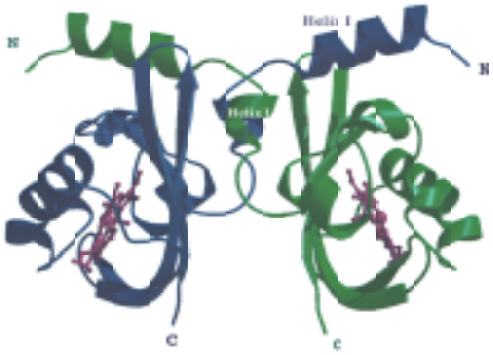

Fig. 4: Overall structure of RmFixLH.

Hideyuki Miyatake, Shin-ichi Adachi and Yoshitsugu Shiro

SPring-8 / RIKEN

miyatake@postman.riken.go.jp

References

[1] H. Miyatake *et al.*, Acta Cryst. D **55** (1999) 1215.

[2] H. Miyatake *et al.* , J. Biol. Chem. **274** (1999) 23176.

[3] H. Nakamura *et al.*, submitted for publication.

[4] H. Miyatake *et al.*, submitted for publication.