BL-NE3A/2019G104

X-ray diffraction experiment for joint X-ray/neutron structure determination of CuNIR

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1 Introduction

Many enzymes of microorganisms play important roles for the nitrogen cycle on the earth. Atmospheric nitrogen gas is converted into chemical species, including ammonia and nitrate, by the nitrogen fixation reaction in microorganisms. The reduction of nitrate in the soil to nitrogen gas is performed by the microbial denitrification process. Copper-containing nitrite reductase (CuNIR) catalyze the reduction of nitrite to nitric oxide, which is a key step in the denitrification process. CuNIR has one type 1 copper (T1Cu), which is the electron acceptor from physiological partner proteins, and has one type 2 copper (T2Cu), which is involved in the nitrite reduction. To understand the molecular mechanism of the nitrite reduction, we have determined neutron crystal structure of CuNIR from *Geobacillus thermodenitrificans* (*Gt*NIR).

2 Experiment

Crystals with a volume more than 1 mm^3 are usually required for neutron diffraction experiment. A large crystal of *Gt*NIR was prepared by a seeding method. Seed crystals of *Gt*NIR were obtained by the hanging-drop vapor-diffusion method at 293 K. The reservoir solution contained polyethylene glycol 4000 as a precipitant. A seed crystal was transferred to a large volume (150 μl) of crystal growth solution consisting of equal volume of protein solution and the reservoir solution. After a large-volume crystal was obtained, the reservoir solution was exchanged to deuterated cryo-protectant solution. Deuterium (^{2}H) reduces background intensities in neutron diffraction data collection compared to hydrogen (^{1}H). The crystal was cooled in a nitrogen-gas stream at 100 K for neutron and X-ray diffraction data collection.

Neutron diffraction data set was collected at the BL03 beamline (iBIX) of J-PARC/MLF. After the neutron diffraction experiment, X-ray diffraction data set was collected using the same crystal at the AR-NE3A beamline of PF-AR. The wavelength of X-rays was set to 1.00 Å and diffraction intensities were measured with Pilatus 2M-F detector. The crystal structure of *Gt*NIR was determined by the molecular replacement method with the crystal structure of the same protein as a search model (PDB ID: 4YSO). The joint X-ray/neutron (XN) refinement was performed using the *Phenix* program.

3 Results and Discussion

The X-ray diffraction data set was obtained at 1.03 Å resolution (Table 1). The crystal belongs to the space group *R*3 (*H*3) with unit cell parameters of *a* = 114.2 Å, *b* = 114.2 Å, *c* = 83.7 Å in *H*3 setting. The joint X/N refinement was performed with the X-ray data up to 1.3 Å resolution because *R*-factors were improved and electron density map showed less noises with the resolution limit. The final model consists of 4952 atoms in the protein molecules and 458 atoms of water molecules including H/D atoms and R_{work}/R_{free} values for the X-ray data were converged to 9.62/11.1%.

The crystal structure analysis clearly indicated that the molecular species coordinating the T2Cu is a deuterated hydroxide (OD^-) molecule. The energetic preference of the coordination of a hydroxide was predicted by the DFT calculation in the nitrite reduction of *Gt*NIR [2]. The hydrogen-bond network, which is connecting the T1Cu and T2Cu sites, forms a stable H-bond deducing from a H/D exchange ratio of the histidine residue (His134) located at the network. The H-bond network has been proposed to consist of a part of the intramolecular electron transfer pathway in CuNIR [3].

Table 1: X-ray diffraction data statistics

Resolution (Å)	42.6-1.03 (1.05-1.03)
Space group	*R*3 (*H*3)
Unit cell *a*, *b*, *c* (Å)	114.2, 114.2, 83.7 (*H*3)
Completeness (%)	99.9 (98.7)
R_{merge} (%)	6.5 (64.1)
$\langle I/\sigma(I)\rangle$	21.2 (3.5)

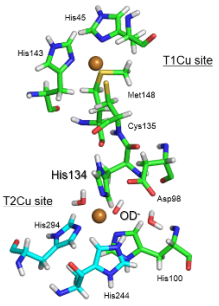

Fig. 1: Active site of GtNIR

Acknowledgement
We thank the PF beamline staff for data collection.

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