MS82.O03

Ultra-high resolution structure of high-potential iron-sulfur protein

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It is important for understanding the electron transfer reaction to include the information about valence shell electrons and hydrogen atoms into crystal structure refinement. High-potential iron-sulfur protein (HiPIP) possesses a Fe4S4 cluster which exhibits +2/+3 redox states and acts as an electron carrier from cytochrome bc1 complex to the reaction center complex in photosynthetic purple bacteria. We have reported the X-ray crystal structure of HiPIP from Thermochtomatium tepidum at 0.72 Å resolution (1). Recently, we have successfully collected 0.48 Å resolution data of HiPIP using high-energy X-rays (31 keV) in BL41XU beamline of SPring-8. We performed multipolar refinement with the MoPro program (2) to consider valence shell electrons in the structure refinement of HiPIP. Refinement of multipolar parameters was applied to atoms of single conformational residues, water molecules with two hydrogen atoms, and the Fe4S4 cluster. After multipolar refinement, the deformation map clearly displays the distribution of valence shell electrons such as lone-pair electrons of carbonyl oxygen atoms, bonding electrons in aromatic rings, and d-orbital electrons of Fe atoms in the Fe4S4 cluster. The deformation map also indicates electrostatic interactions between the S atoms of Fe4S4-(Cys-Sγ)4 and protein environment. In addition, we performed preliminary neutron diffraction experiment at iBIX beamline of Japan Proton Accelerator Research Complex (J-PARC) and observed diffraction spots up to 1.17 Å resolution using HiPIP crystal with the size of 2.3 mm3. In the multipolar refinement, the positions of hydrogen atoms were fixed to the standard bond distances derived from neutron crystal structures of small molecules and atomic displacement parameters of hydrogen atoms were constrained to 1.2 or 1.5 fold of their root atoms. Therefore, a high resolution neutron structure of HiPIP will improve the results obtained from the multipolar refinement.

[1] *K. Takeda et al., J. Struct. Biol., 2010, 169, 135-144,* ***[2]*** *B. Guillot et al., J. Appl. Cryst., 2001, 34, 214-223*

Keywords: multipolar refinement, Fe4S4 cluster