Ultra-high resolution crystal structure and charge density analysis of HiPIP in bacterial photosynthesis

More than 110,000 protein structures determined by X-ray crystallography are now available in the Protein Data Bank (PDB). Most of these deposited structures fall in the range of conventional resolution of between 1.5 Å and 3.0 Å. In this resolution range, structures provide information on the folding of protein molecules and the architecture of their active sites but are insufficient in their precision and quantity to elucidate the molecular mechanism of protein functions directly from structural information. However, the recent technological development of synchrotron radiation has enabled us to obtain high-quality X-ray diffraction data at ultra-high resolutions to extract more detailed structural information such as the hydrogen position and charge density distribution in protein crystal structures. We have determined the crystal structure of a high-potential iron-sulfur protein (HiPIP) from a thermophilic bacterium at 0.48 Å resolution and performed charge-density analyses, where the distributions of valence electrons in metalloproteins were clearly visualized for the first time [1]. HiPIP is an electron-transfer protein involved in the primary process of bacterial photosynthesis, where sunlight energy is converted to chemical energy (Fig. 1(a)). This protein carries electrons from the cytochrome bc_1 complex to the photosynthetic reaction center to reduce the photo-oxidized bacteriochlorophyll dimer (special pair) in the reaction center [2].

Diffraction data from crystals of HiPIP were collected at SPring-8 **BL41XU** with high-energy X-rays (λ = 0.45 Å, E = 27.6 keV) (Fig. 1(b)). The structure was successfully refined at 0.48 Å resolution which is, to date, one of the highest resolutions among all the protein structures deposited in PDB. The structure was initially refined with the conventional independent spherical atom model (ISAM) parameters, where residual electron densities were observed around each atom. Further refinement was performed using the multipolar atomic model (MAM) [3] in order to obtain charge-density information. The final R factor is 7.16% (R_{free} = 7.80%) for 301,119 reflections (PDB ID: 5D8V).

The Fe_4S_4 cluster is coordinated by four cysteine residues and is further surrounded by aromatic residues (Fig. 1(a)). Several distorted peptide bonds largely deviated from the planar configuration are observed mostly in the proximal region of the Fe_4S_4 cluster. Valence electrons of the polypeptide portion are visualized for the side and main chains of the polypeptide portion in the residual maps refined by the ISAM and in the deformation maps refined by the MAM.

The charge-density analysis also indicates a nonspherical distribution of the electron density around the Fe_4S_4 cluster (Fig. 2(a)). The Fe 3d-orbital electron densities are observed around the Fe atoms, whereas the bridging S and Cys-S_γ atoms are surrounded by diffused S 3p-orbital electrons. The Fe_4S_4 cluster consists of two subclusters: subcluster 1 composed of FE1, FE2, S3, S4, Cys43-S_γ and Cys46-S_γ and subcluster 2 composed of FE3, FE4, S1, S2, Cys61-S_γ and Cys75-S_γ. The Fe-S bonds within the subclusters

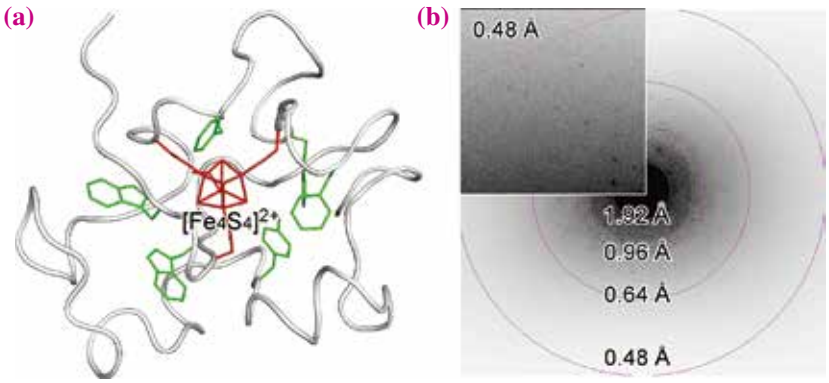

Fig. 1. Structure of high-potential iron-sulfur protein (HiPIP) and an ultra-high resolution diffraction image from its crystals. **(a)** Overall view shown as a tube model, where the iron-sulfur cluster and aromatic residues are represented as red and green sticks, respectively. HiPIP from *Thermochromatium tepidum* is composed of 83 amino acid residues and has an Fe_4S_4 cluster. **(b)** Diffraction image obtained at BL41XU, in which diffraction spots are observed at 0.48 Å resolution.

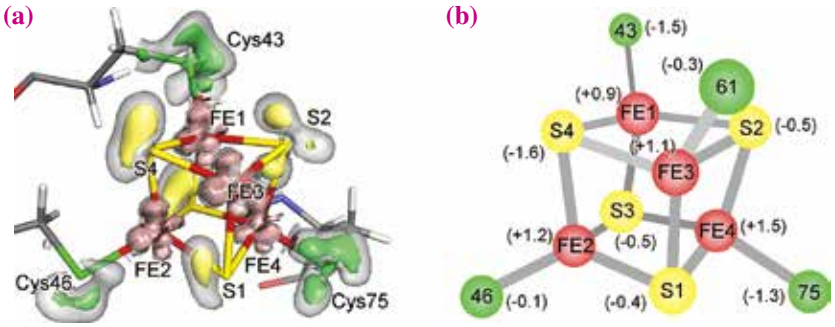

Fig. 2. Structure of the Fe_4S_4 cluster in HiPIP. **(a)** Deformation map of the Fe_4S_4 cluster represented as gray isosurfaces (+0.1 e/Å^3), with red, yellow and green (+0.3 e/Å^3) isosurfaces representing iron, cluster-sulfur and cysteine-sulfur atoms, respectively. **(b)** Schematic representation of the cluster in which atomic charges are indicated in parentheses near the corresponding atoms.

are long, whereas those bridging two subclusters are short in the Fe_4S_4 cluster. The overlaps between the Fe 3*d* and S 3*p* orbitals are generally smaller for shorter Fe-S bonds and larger for longer bonds. The atomic charges for the $Fe_4S_4(Cys\text{-}S_\gamma)_4$ moiety were derived by using the atoms in molecules (AIM) theory [4] (Fig. 2(b)). The atomic charge of FE1 is lower than those of other iron atoms and that of S4 is also lower than those of other bridging S atoms. The atomic charges of Cys43-S_γ and Cys75-S_γ are lower than those of Cys46-S_γ and Cys61-S_γ. The total atomic charges for subclusters 1 and 2 are –1.62 and +0.15, respectively, indicating that subcluster 1 is important for storing electronic charges.

It was found that the valence electrons of S atoms of $Fe_4S_4(Cys\text{-}S_\gamma)_4$ interact with H atoms in the charge-density map (Fig. 3). The atomic charges of S atoms correlate with the interaction between valence electrons and H atoms. Among the four bridging S atoms, S4, whose atomic charge is the largest, has no such interactions but the other three S atoms with smaller charges must have significant interactions. These findings imply that charge transfer from S to H atoms is involved in the reduction of the negative charges of S through the interactions.

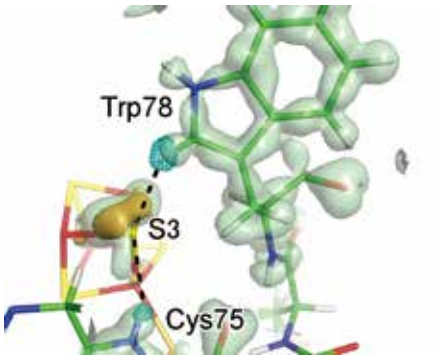

Fig. 3. Interactions between the Fe_4S_4 cluster and the protein environment. Deformation electron densities around S3 of the Fe_4S_4 cluster are presented as light green and pale yellow isosurfaces at contour levels of +0.1 and +0.3 e/Å^3, respectively. Cyan meshes show omit-map densities of the amide H of Cys75 and a H atom of Trp78 at a contour level of 3σ.

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