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X-ray Crystal Structure Analyses and QM/MM Calculations of Pseudoazurin Met16 Variants

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1 Introduction

Noncovalent interactions (NCI) with the interaction energy, < 10 kJ/mol have been discussed as the important factor in many biological molecules[1]. The effects of NCI at the second coordination sphere were rationally studied in a blue copper protein, pseudoazurin (PAz) Met16 variants [2-3]. In this experiment, the combined study of high-resolution crystal structure analyses and QM/MM calculations of PAz Met16 variants unveiled the structural and energetic features of NCI at the second coordination sphere of the active site.

2 Experiment

The crystals of oxidized Met16Phe, Met16Val, and Met16Ile PAz were obtained by the hanging drop vapor diffusion method. The X-ray diffraction experiments of PAz were carried out at the beam line 5A of Photon Factory and NW12A of Photon Factory Advanced Ring. Computational models created from 1.1 Å crystal structure of WT PAz (PDB ID: 4YL4) and methods were validated using the obtained crystal structures of Met16 variants.

3 Results and Discussion

The high-resolution crystal structure analyses showed the maximum resolution of Met16Phe, Met16Leu, Met16Val, and Met16Ile PAz were 1.40, 1.19, 1.05 and 1.00 Å, respectively. The crystal structures identified S-π/CH-π interaction in wild type PAz, face-to-face/face-to-edge π-π interaction in Met16Phe variant, double CH-π interaction in Met16Leu, and single CH-π interaction in Met16Val and Met16Ile variants in the second coordination sphere (Fig. 1). The computational chemistry investigations were employed based on the X-ray crystallographic structural data of Met16X PAz to add more detailed knowledge into the noncovalent weak interactions at the second coordination sphere of protein. The noncovalent interaction at the second coordination sphere were validated using the NCI plot analyses in Fig. 2. Furthermore, the interaction energy calculated from QM/MM structures showed the clear correlations with the spectroscopic and electrochemical properties. In conclusion, the high-resolution X-ray crystal structure analyses and computational calculations of Met16 PAz variants approved that the noncovalent weak interactions, π-π, S-π, and CH-π interactions, in the second coordination sphere provide the good control of metalloprotein active site. The knowledge of NCI at the active site on the physical properties is believed to apply the precise design of industrially useful enzymes and protein drugs through the appropriate selection of the weak interactions.

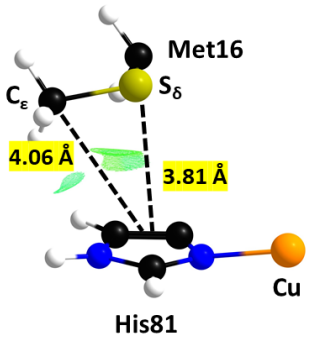

Fig. 2. NCI isosurface of S-π interaction in WT PAz.

References

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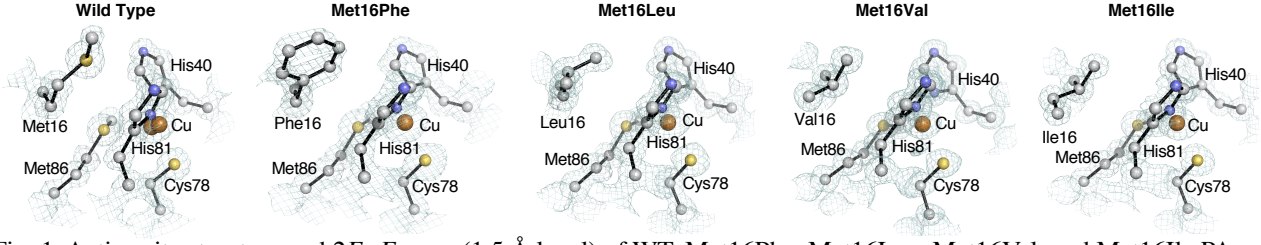

Fig. 1. Active site structure and $2F_o$-F_c map (1.5 Å level) of WT, Met16Phe, Met16Leu, Met16Val, and Met16Ile PAz.