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X-ray Crystallographic Analysis of M16F/T36K Double Mutant of Pseudoazurin

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1 Introduction

The structure and function of active sites in protein is determined by not only the immediate molecular environment but also the outer sphere interactions, such as electric dipole, hydrogen bond and so on. In metalloproteins, the regulations of Cu site properties through the modifications of hydrogen bonds or hydrophobicity of coordination environment were reported by Lu and his co-workers [1]. We also reported the importance of hydrogen bond network for the electronic structure of heme Fe in cytochrome *c*' [2]. Non-covalent weak interaction due to the dispersion force between amino acid residues are also known to attends the structure-and-function relationship of proteins, however, the rational design of active site properties depends on the outer sphere environment involving the weak interactions, which have never been reported to date.

Pseudoazurin (PAz) has the redox-active Cu site for a biological electron transfer. The second-sphere non-covalent weak interaction in the PAz has been studied by structure analysis, several spectroscopic methods, electrochemistry, and theoretical approach [3]. The high resolution X-ray crystal structure analysis of M16F/T36K double mutant PAz was performed at Photon Factory to know of the additivity of spectroscopic/electrochemical properties for each single mutation, M16F and T36K.

2 Experiment

The protein of M16F/T36K variant was expressed in *E. coli* and purified. The crystal of M16F/T36K variant was obtained by the hanging drop vapour diffusion method. The single crystal X-ray diffraction data for the M16F/T36K variant were collected at beam line NW 12A (PF-AR). The temperature of crystal was 100 K. The space group of M16F/T36K variant was *C*2 space group, and the cell parameters were a = 93.2 Å, b = 41.2 Å, c = 34.8 Å, $\alpha = \gamma = 90.0^\circ$, $\beta = 98.7^\circ$. The structure of M16F/T36K variant was refined at 1.19 Å resolution. (PDB ID: 5XMO)

3 Results and Discussion

The structure of M16F/T36K PAz at 1.19 Å resolution demonstrated the co-existence of two Cu position, which two conformers were assigned to the axial and rhombic structure in the Wild Type (WT) PAz as we reported previously [4]. Several structural changes are recognized at the mutated amino acid residues in the outer sphere region of M16 and T36 residues. The replacement of M16 to phenylalanine provides π-π interaction with copper coordinated H81. The structure in the double mutant, M16F/T36K is consistent with M16F single mutant [5]. Interestingly the mutation of T36 to lysine residue gave the structural rearrangement of hydrogen-bonding networks around H6 (Figure 1). The hydrogen bond between H6 and T36 recognized in the structure of WT protein is disappeared in the double mutant protein. A new hydrogen bond between H6 and E4 is observed instead of the H6-T36 hydrogen bond in the M16F/T36K. The other rearrangements of hydrogen bonds are also induced by the movement of P35, I34, and F33 of the double mutant PAz. The relevant studies for the additivity of spectroscopic/electrochemical properties for the double mutant are currently in progress based on the X-ray crystal structure analysis of M16F/T36K double mutant.

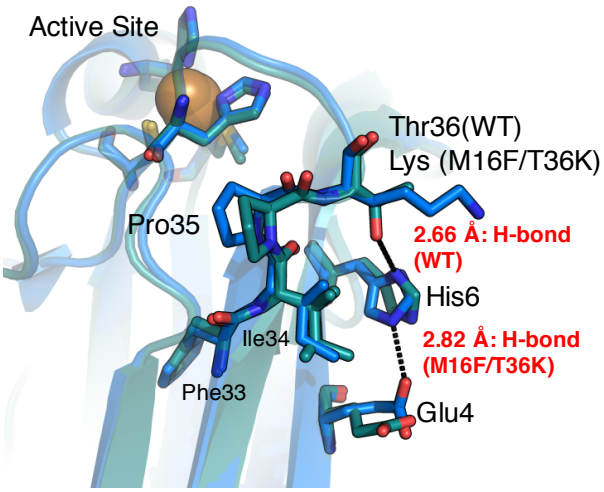

Fig. 1: The rearrangement of hydrogen bond around His6 and flipping of Pro35 between wild type (green) and Met16Phe/Thr36Lys (blue).

References

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