Regulation of the Structure of Blue Copper Active Site through the Hydrogen Bonding between His6 and Thr36 residues: Spectroscopic and Electrochemical Properties of Pseudoazurin Thr36Lys Mutant

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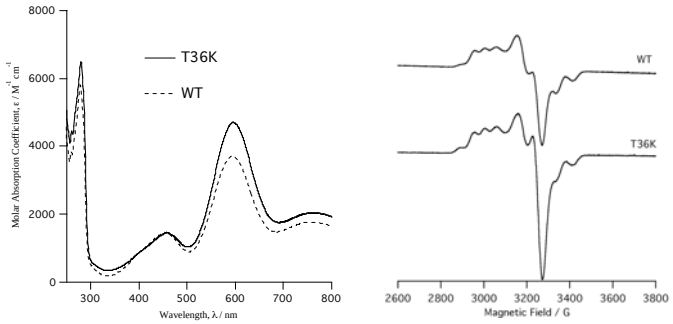
Noncovalent weak interactions play important roles in biological systems. Recently, the structures of oxidized and reduced forms of a blue copper protein, pseudoazurin (PAz) were reported [1]. The structure analysis of PAz indicated the rearrangement of hydrogen bonding around a non-coordinated histidine residue, His6. In the structure of reduced PAz, the side chain of a threonine residue, Thr36, makes additional hydrogen bonds with the imidazole nitrogen of His6. The protonation / deprotonation of His6 is considered to influence the active site structure and properties of PAz [2].



To investigate the effect of the hydrogen bonding around the His6 residue, Thr36Lys mutant of PAz has been constructed and characterized. In the electronic absorption spectrum of Thr36Lys mutant, the most intense absorption band is shifted to 597 nm, and the molar absorption coefficient increases to 4700 M^{-1} cm^{-1}. The EPR spectrum of Thr36Lys mutant displayed a more axial signal pattern accompanied by the enhancement of the fourth signal in the $A_{//}$ region. The redox potential of Thr36Lys mutant was estimated to be 285 mV vs. NHE, which is slightly higher than that of WT protein (260 mV vs. NHE).

References.

1. T. Inoue, N. Nishio, S. Suzuki, K. Kataoka, T. Kohzuma, and Y. Kai, *J. Biol. Chem.*, **274**, 17845(1999).
2. K. Sato and C. Dennison, *Biochemistry*, **41**, 120(2002).