Regulation of the Electronic Structure of Blue Copper Active Site from the Second Sphere Coordination: Spectroscopic and Electrochemical Properties of Pseudoazurin M16X Mutants

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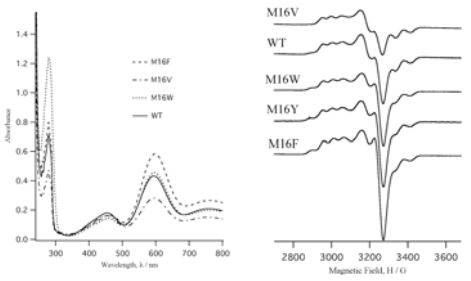
Noncovalent weak interactions play important roles in biological systems. Very recently, we reported the structure and reactivity of the M16F mutant of the blue copper protein pseudoazurin (PAz) to investigate the effects of the π-π interaction observed in the unusual structure and reactivity of fern plastocyanin [1, 2, 3]. The Met16 substituted mutants of PAz, in which several alkyl and aromatic groups are introduced close to the imidazole of the H81 ligand, have been constructed and characterized in order to probe the effects of the indirect weak interaction on the structure and function of the active site. Electronic absorption spectra of M16Y, M16W, and M16F mutants indicated the smaller ratio of A_{460}/A_{598} = ~0.3 as compared to that of wild type PAz, A_{460}/A_{594} = 0.46. EPR spectra of the M16F, M16Y and M16W mutants indicated the enhancement of the axial signal contribution. The EPR spectrum of M16V mutant clearly showed the loss of a small fourth signal in the $A_{//}$ region, and the EPR spectrum displayed completely rhombic signal pattern. The redox potential of M16Y, M16W and M16V mutants were evaluated to be 315, 291 and 278 mV vs. NHE (pH 7), respectively.



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