1P061 Spectroscopic and Electrochemical Studies of Pseudoazurin Met16X Variants
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A. *cycloclastes* pseudouazurin (PAZ) functions as an electron donor to the nitrite reductase. PAZ has a single copper atom at the active site, and the copper atom is buried approximately 5 Å from the protein surface. The structure of active center has a distorted tetrahedral geometry, with two histidines (His40, His81), one cysteine (Cys78), and one methionine (Met86) as ligands. In the structure of PAZ, the solvent exposed His81 seems to interact with the side chain group of Met16 within a van der Waals radius. Very recently, we reported the structure and properties of the Met16F PAZ mutant to investigate the effects of the *π*-*π* interaction observed in the ferr plastocyanin, which has unusual structure and reactivity.

Here we would like to report that the effect on the electronic structure of PAZ by the substitution of Met16 residue to several amino acids having alkyl group in the side chain. Electronic absorption spectra of WT, M16A, M16V, and M16L mutant proteins showed the absorbance ratios at 460 nm and 600 nm were 0.46, 0.60, 0.57 and 0.43, respectively. The ratio values are significantly higher except M16L variant than that of WT PAZ, and the higher values reflects increasing of the rhombic structure population. The EPR spectrum of M16V mutant clearly showed the loss of a small fourth signal in the A/region, and the EPR spectrum of M16V displayed completely rhombic signal pattern. The redox potential of M16A, M16V, and M16L mutants were evaluated to be 271, 278 and 289 mV vs. NHE (pH 7.0), respectively.

1P062 Aromatic Ring Stacking Interaction in a Blue Copper Protein, Pseudoazurin
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Pseudoazurin (PAZ) is a type 1 blue copper protein, which functions as an electron carrier in denitrifying bacteria. PAZ possesses a single copper atom at the active site and gives an intense absorption band due to a *S*(Cys) - Cu charge transfer transition at 594 nm (ε = 3700 M⁻¹ cm⁻¹) and a narrow hyperfine coupling constant in the EPR spectrum of the oxidized form [1]. The copper atom of PAZ is coordinated by two histidines (His40 and His81), a cysteine (Cys87) and a methionine (Met86) with a distorted tetrahedral geometry [2]. In the structure of PAZ, the solvent exposed His81 interacts with the side chain of Met16. The Met16 residue was substituted by Tyr and Phe, and the protein variants were expressed in *E. coli* and purified to investigate the *π*-*π* interaction effect on the electronic structure of the PAZ active site.

UV resonance Raman spectra of Met16Tyr and Met16Phe mutants in D_2O were measured by the excitation at 244 nm. A Raman band (v = N(C=N) of imidazole) of copper coordinated histidine ligands were observed at 1385 cm⁻¹ in the oxidized form of WT protein. The Raman band is shifted to ~4 cm⁻¹ higher frequency for the *π*-*π* interaction introduced Met16Tyr and Met16Phe variants.


1P063 Comparative analysis of ribosome atomic structures deduced computationally from EM images and X-ray structures
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Single particle imaging using an electron microscope (EM) is becoming one of the major methods in determining the three-dimensional (3D) structures of biological supramolecules, because the EM method can obtain nano scale molecular structures with little restriction of sample preparation. The resolution of an EM image is, however, in many cases much lower than the atomic level and therefore 3D structures of the supramolecules at atomic resolution can be obtained by fitting the 3D structures of constituent molecules determined by X-ray crystallography into the EM density maps. Usually the fitting is done manually without systematically accounting for atomic collisions and bond stresses in the structure.

We are developing an EM density-fitting refinement method to improve the modeled 3D structure of supramolecules. Our method first uses rigid body fitting to carry out initial fitting, and then uses a molecular dynamic simulation, in which a target function together with the standard all-atom energy function is used to constrain the atomic structure into the EM map.

Here we report the application of this method to the Thermus thermophilus 70S ribosome. In this study we have fit the X-ray crystallographic structure of the ribosome into multiple EM images which show the ribosome in different stages of translation. The refined structures are compared and as a result differences in structures are observed. The differences are thought to indicate the functional movements of the ribosome.

1P064 Comprehensive analysis of the MHC-I binding of leukocyte immunoglobulin-like receptors using surface plasmon resonance technique
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Leukocyte immunoglobulin (Ig)-like receptor (LILR) family including activating, inhibitory, and soluble form receptors is broadly expressed on leukocytes. *Group 1* LILRs (LILRA1/A2/A3/B1/B2) have over 70% sequence similarity to LILRB1 and B2, which bind to a wide range of human major histocompatibility complex (MHC) class I molecules (MHC1). On the other hand, *Group 2* LILRs (LILRA3/B3/B4/B5) show less than 60% sequence similarity. Preliminary cellular studies have shown that, except for LILRB1, B2, and A1, other LILRs do not bind some MHC1s. In this study, we examined whether LILR molecules can bind to classical and nonclassical MHC1s or not by using surface plasmon resonance. The soluble N-terminal two Ig-like domains (D1D2) of LILRs, which correspond to the ligand binding domains of LILRB1 and B2, were expressed by *E. coli* as inclusion bodies and successfully refolded. A wide range of C-terminal biotinylated MHC1s (HLA-A, B, C, E, and G) were immobilized on the sensor chip, and each LILRD1D2 was flowed over. All Group2 LILRs did bind to any tested MHC1s consistent with the cellular analyses. As for Group1 LILRs, unexpectedly, LILRA2 did not show any MHC1 binding. On the other hand, LILRA3, which is an only soluble member of LILR family, was able to bind to HLA-G specifically, in contrast with broad MHC1 recognition of LILRB1 and LILRB2. Further analyses of binding properties of the LILRA3-HLA-G recognition are underway.