II-E Structure and Function of Metalloproteins and Its Molecular Design

Metal ion is a common cofactor that is crucial for active centers of proteins involved in many biologically important processes in cells, and a relatively small number of metal-based prosthetic groups are utilized to serve numerous and diverse chemical functions. A typical metal-based prosthetic group, which represents a fascinating example in this respect, is heme. Heme promotes a variety of functions, such as dioxygen storage, activation of small molecules, electron transfer reactions, and sensing gaseous molecule. In the field of protein design and engineering, hemoproteins also make particularly attractive targets. There are many reasons for this, including the exciting possibility of engineering protein-based molecules with useful catalytic, electronic or optoelectronic properties. Based on various kinds of spectroscopies, we have functionally and structurally characterized some hemoproteins including newly identified heme-regulated proteins, and designed hemoproteins showing improved activities and new functions.

II-E-1 Structural and Functional Characterization of "Laboratory Evolved" Cytochrome P450cam Mutants Showing Enhanced Naphthalene Oxygenation Activity

MATSUURA, Koji[1]; TOSHA, Takehiko[1]; YOSHIOKA, Shiro[1]; TAKAHASHI, Satoshi[1]; ISHIMORI, Koichiro[2]; MORISHIMA, Isao[1]
([1]Kyoto Univ.; [2]IMS and Kyoto Univ.)

[*Biophys. Biochem. Res. Commun.* **324**, 1095 (2004)]

To elucidate molecular mechanisms for the enhanced oxygenation activity in the three mutants of cytochrome P450cam screened by 'laboratory evolution' (H. Joo, Z. Lin and Z. F. H. Arnold, *Nature* **399**, 670–673 (1999)), we purified the mutants and characterized their functional and structural properties. The electronic absorption and resonance Raman spectra revealed that the structures of heme binding site of all purified mutants were quite similar to that of the wild-type enzyme, although the fraction of the inactivated form, called "P420", was increased. In the reaction with H_2O_2, only trace amounts of the naphthalene hydroxylation product were detected by gas chromatography. We, therefore, conclude that the three mutants do not exhibit the significant changes in the structural and functional properties from those of wild-type P450cam except for the stability of the axial ligand in the reduced form. The enhanced fluorescence in the whole-cell assay would reflect the enhancement in the oxygenation activity below the detectable limit of the gas chromatography and/or contributions of other reactions catalyzed by the heme iron.

II-E-2 Spectroscopic Characterization of Heme Binding to the Heme Regulatory Motif (HRM) in the Bacterial Iron Response Regulator Protein

ISHIKAWA, Haruto[1]; TAKAHASHI, Satoshi[1]; HORI, Hiroshi[2]; O'BRIAN, Mark R.[3]; MORISHIMA, Isao[1]; ISHIMORI, Koichiro[4]
([1]Kyoto Univ.; [2]Osaka Univ.; [3]New York State Univ.; [4]IMS and Kyoto Univ.)

[*J. Biol. Chem.* Submitted]

The heme regulatory motif (HRM) is a common and crucial amino acid sequence for the heme binding in heme-regulated proteins, but the structural characterization of the heme binding to HRM has not yet been extensively accomplished. The bacterial iron response regulator (Irr), controlling the heme biosynthesis by degrading itself in the presence of iron, has one HRM in the sequence. Although the absorption spectrum of Irr in the presence of ferric heme was quite unusual in that the Soret peak was broad with highly blue-shifted and suggestive of the dissociation of the axial ligand from protein, the EPR signals (g = 2.52, 2.29, 1.90, g = 4 to 8) and the ν_3 line at 1491 cm^{-1} in the resonance Raman spectrum were characteristic of Cys-ligated hemes. By the mutation of ^{29}Cys to Ala, the EPR signals and the ν_3 line from the Cys-ligated heme diminished, confirming that ^{29}Cys is the axial ligand for ferric heme bound Irr. In sharp contrast to the ferric heme bound Irr, the spectroscopic features of the ferrous heme bound ^{29}Cys → Ala mutant are quite similar to those of wild-type Irr. In addition, the correlation of the stretching modes of ν(Fe–CO) and ν(FeC–O) for ferrous CO heme bound Irr indicates that the axial ligand trans to CO is histidine. The reduction of the heme iron, therefore, replaces axial ^{29}Cys with histidine. These results provide the first detailed spectroscopic characterization for the heme binding to HRM and provide evidence for redox-dependent axial ligand exchange in Irr.