bound species appeared. A strong signals of hydroperoxo-intermediate ($g=2.30, 2.17$) were observed using uncoupled system with T252A mutant P450cam. This intermediate decreased and the camphor bound species increased by annealing. We could confirm all intermediate species as proposed previously in the actual reaction cycle.

1P-080 デオキシヘモグロビンの電子状態解明に向けた連続可変多周波 EPR 共振器の開発

Development of a continuously frequency-variable EPR resonator to elucidate the electronic structures of deoxy-hemoglobin

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Metalloproteins which have transition metal ions play a critical role in the biological functions. The biological functions of hemoglobin (Hb) and myoglobin (Mb) which are the most fundamental metalloproteins are profoundly related to the electronic structures of their metal centers. Thus, it is very important to clarify the electronic structures of metalloproteins. Recently, we succeeded in analyzing the EPR spectra of Mn(III)Mb ($Mn^{3+}, S=2$) by using our high field (0-14 T) and multi-frequency (34-122 GHz) EPR system with cylindrical resonators. But for deoxy-Hb ($Fe^{2+}, S=2$), we could not analyze the EPR results due to the limitation of the resonant frequency for the cavity resonator. Whereat, we have developed a new Fabry-Perot Resonator (FPR) with which we can do broadband and continuous frequency measurements without changing the sample. Several FPRs for EPR have been reported so far. However, our FPR has a newly developed matching system to observe broad signals very precisely. It consists of a wire-grid mirror and a rectangular waveguide. The best matching condition can be easily achieved by simply rotating the wire-grid mirror against the waveguide. To realize a broadband (30-300GHz and even at higher frequencies) FPR with high sensitivity, we have designed the FPR with an appropriate geometry for this frequency range and have simulated its performance. We are now fabricating and testing our new FPR. Several EPR results of deoxy Hb and the FPR setup details will be reported.

1P-081 ポルフィリン側鎖のヘムの電子構造への影響に関する理論的研究 Density Functional Study of Roles of Porphyrin Ring in Electronic Structures of Heme

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Heme is an ubiquitous cofactor in biomolecules and plays a central role in biology, especially in electron transfer and enzyme catalysis. There are many types of heme, which have several heme side chains such as propionate groups. The differences in axial ligands and side chains yield many important functions of heme. In this study, the roles of porphyrin ring such as heme porphyrin ring distortion, heme peripheral substitution, and protonation of propionyl group of heme, in the charge density differences and redox potentials of heme have been studied by using density functional theory with polarizable continuum model.

B3LYP and PW91 exchange-correlation functionals were employed on five models of bis-histidine ligated heme, heme *a* model, CcO heme *a* model, heme *b* model, heme *o* model, and deprotonated heme *a* model, to elucidate the roles of heme porphyrin ring in the charge density difference and redox potential in the redox reaction.

Our computational data show that heme is robust over heme peripheral substitutions and heme porphyrin ring distortion in electron transfer and that the redox potential of heme is determined and regulated by the heme substituents and the subtle balance of the properties of surrounding protein: the distortion of porphyrin ring, the strength and number of hydrogen bonds, and the electrostatic field of protein and that heme has a wide range of redox potential in various proteins. These characteristics enable heme to generate diverse biological functions and to widely distribute over the biological systems as electron mediators.

1P-082 ヒト由来インドールアミン 2,3-ジオキソゲナーゼにおけるヘム近傍アミノ酸残基の役割と基質阻害機構

Role of the Heme Environmental Amino Acid Residues and Mechanism of the Substrate Inhibition in Human Indoleamine 2,3-Dioxygenase

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Indoleamine 2,3-dioxygenase (IDO) which contains iron protoporphyrin IX, *i.e.* heme *b*, as the prosthetic group in the active site is ubiquitously expressed the mammals except for liver. IDO catalyzes the cleavage of the pyrrole ring of L-tryptophan by insertion of molecular oxygen and produces *N*-formylkynurenine, which is the initial and rate-limiting step of L-tryptophan catabolism in the kynurenine pathway. We previously reported the structure of recombinant human IDO (hIDO) and proposed that the precise ternary complex, substrate-ligand-enzyme, was essential for the catalytic reaction (1). However, the detail of the

reaction mechanism has not been understood yet.

In this study, we performed mutation analysis on the amino acid residues at loop region and the aromatic amino acid residues nearby active site that are conserved in IDO family. Comparison of kinetic parameters, dissociation constants for L-tryptophan and resonance Raman spectra for CO derivative of wild type and these mutants indicates that the aromatic amino residues do not play a essential role in the formation of ternary complex. Instead, amino acid residues at loop region, G261, S263 and A264, play a critical role in dioxygenase reaction.

We observed the substrate inhibition for wild type hIDO more than about 150 μ M L-tryptophan at pH 6.5. The analysis of the structure of the product at various concentrations of L-tryptophan by mass spectroscopy revealed two kinds of products.

(1) H. Sugimoto et al. Proc. Natl. Acad. Sci. USA 103 (2006) 2611

1P-083 チトクロム酸化酵素休止酸化型の 1.4 Å 分解能 X 線構造解析 X-ray structural analysis of cytochrome c oxidase in the resting oxidized state at 1.4 Å resolution

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Cytochrome c oxidase (CcO) in the respiratory chain catalyzes the oxygen reduction coupling to the proton pump across the membrane. To understand mechanism of energy transduction in CcO, the atomic structures have to be determined with high accuracy. Especially, it is important to identify accurate structural change at the catalytic site and protonation state in the proton transfer pathway. For these purpose, we are currently collecting X-ray diffraction data of bovine CcO crystals at 1.4 Å resolution. The crystal is formed by CcO in the resting oxidized state, which is obtained after isolation from the mitochondrial membrane and stable under aerobic environment. By using two types of CcO crystals with different crystal packing, the diffraction data were collected at BL44XU and BL41XU in SPring-8. To reduce the influence of X-ray irradiation on the structure, the measurements were performed under cryo-helium stream at 40 K and the X-ray dose was reduced by merging the data from many crystals. To improve the accuracy of the data, the redundancy of the diffraction was increased by collecting total 5,455 and 21,000 images from two types of crystals, respectively. The electron density map obtained by processing 1,650 images revealed binding manner of the ligand molecule in the resting oxidized catalytic site. Furthermore, multi-conformation of amino acid side chain identified near the catalytic site suggests a role as an exit pathway of the catalytic products.

1P-084 多剤排出トランスポーターAcrBの分子動力学シミュレーション All-atom molecular dynamics simulation of bacterial multidrug efflux transporter AcrB

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Multidrug efflux transporters cause for antimicrobial resistance in the chemotherapy of cancer and antibiotic treatment of numerous different bacterial infections. In *E. coli*, it is known that the tripartite multidrug efflux system (AcrB/AcrA/TolC) exists, and AcrB resides in the inner membrane region and take part in substrate recognition and energy transduction for drug export through proton transfer. Recently, x-ray structures provided that AcrB forms trimeric protein where each subunit is different conformation, "binding state", "extrusion state" and "access state". Especially, only extrusion state subunit has different side chain conformation of residues (Asp407, Asp408 and Lys940), which are essential for proton translocation. These results suggest that drugs are exported by a three-step structural change involved in proton motive force. However, the structural change process which involved in proton motive force is still unclear. In the present study, we performed 100 ns all-atom molecular dynamics (MD) simulations of AcrB-membrane-water system and analyzed the structural change mechanism among three subunits. In the MD simulation, in order to induce the structural change of each subunit, AcrB binds no drugs and all of residues essential for proton translocation were not protonated. During the 100 ns MD simulation, the global structures of each subunit were conserved. However, in extrusion state, the side chain conformation of Asp407, Asp408 and Lys940 changed to those of other subunits. This result suggests that the local structural transition from extrusion state to another state occurred. In this presentation, we will show the comparison of dynamics and water accessibility among each state subunit and discuss the structural change and proton transfer mechanism.

1P-085 膜活性タンパク質の新規設計

De novo design of membrane-active proteins

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Though membrane proteins perform important functions in biological systems, we often encounter experimental difficulties compared to the case of water-soluble proteins because of their insolubility and complexity. Therefore, *de novo* design